

Diagnosis, epidemiology and control of soil-transmitted helminth infections in Zanzibar, Tanzania

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Table of contents

1. Acknowledgements	17
2. Summary	21
3. Zusammenfassung	25
4. Muhtasari	29
5. Introduction	33
5.1. Helminth infections	33
5.1.1. <i>Life cycle and biology of soil-transmitted helminths</i>	34
5.1.2. <i>Morbidity and burden of soil-transmitted helminthiases</i>	35
5.1.3. <i>Soil-transmitted helminth infections in Zanzibar</i>	36
5.2. Control of soil-transmitted helminth infections.....	37
5.2.1. <i>Large-scale deworming</i>	37
5.2.2. <i>Anthelmintic drug efficacy</i>	38
5.2.3. <i>Health education</i>	40
5.2.4. <i>Provision of adequate sanitation and clean water</i>	41
5.2.5. <i>Helminth control programmes in Zanzibar</i>	43
5.2.6. <i>Drug efficacy in Zanzibar</i>	44
5.2.7. <i>Health education in Zanzibar</i>	45
5.2.8. <i>Environmental sanitation in Zanzibar</i>	45
5.3. Diagnosis of soil-transmitted helminth infections	45
5.4. References	48
6. Identified research needs	59
7. Goals.....	59
7.1. Specific objectives	59
8. Study sites.....	61
9. Diagnosis of soil-transmitted helminths in the era of preventive chemotherapy: effect of multiple stool sampling and use of different diagnostic techniques.....	63
9.1. Abstract	64
9.2. Author Summary	65
9.3. Introduction.....	66
9.4. Materials and methods	67
9.4.1. <i>Study area and population</i>	67
9.4.2. <i>Field and laboratory procedures</i>	68
9.4.3. <i>Data management and statistical analysis</i>	69

9.4.4. <i>Ethical considerations and treatment</i>	70
9.5. Results	70
9.5.1. <i>Study cohort</i>	70
9.5.2. <i>Parasitological findings, stratified by diagnostic method</i>	72
9.5.3. <i>Infection characteristics</i>	74
9.5.4. <i>Performance of the diagnostic methods</i>	76
9.6. Discussion.....	78
9.7. Acknowledgments.....	81
9.8. References	82
9.9. Translation of abstract into German.....	86
10. A single FLOTAC is more sensitive than triplicate Kato-Katz for the diagnosis of low-intensity soil-transmitted helminth infections	87
10.1. Summary.....	88
10.2. Introduction.....	89
10.3. Materials and methods	90
10.3.1. <i>Study area and population</i>	90
10.3.2. <i>Ethical considerations and anthelmintic treatment</i>	90
10.3.3. <i>Field and laboratory procedures</i>	91
10.3.4. <i>Statistical analysis</i>	92
10.4. Results	93
10.4.1. <i>Operational results</i>	93
10.4.2. <i>Comparison of methods</i>	94
10.5. Discussion.....	100
10.6. Acknowledgments.....	102
10.7. References	104
11. FLOTAC: a promising technique for detecting helminth eggs in human faeces[☆] 107	
11.1. Summary.....	108
11.2. Introduction.....	109
11.3. Methods	109
11.4. Results	110
11.5. Discussion.....	112
11.6. Acknowledgements	113
11.7. References	114

12. Diagnostic Accuracy of Kato-Katz and FLOTAC for Assessing Anthelmintic Drug Efficacy	115
12.1. Abstract	116
12.2. Author Summary	117
12.3. Introduction.....	118
12.4. Materials and methods	119
12.4.1. <i>Ethics statement</i>	119
12.4.2. <i>Study area and population</i>	120
12.4.3. <i>Field and laboratory procedures</i>	120
12.4.4. <i>Statistical analysis</i>	122
12.5. Results	124
12.5.1. <i>Operational results</i>	124
12.5.2. <i>Method comparison: diagnostic sensitivity</i>	126
12.5.3. <i>Observed prevalence and infection intensities</i>	126
12.5.4. <i>Estimated CR and ERR</i>	127
12.6. Discussion.....	135
12.7. Acknowledgments.....	139
12.8. References	140
12.9. Translation of abstract into German.....	144
13. Spatial distribution of soil-transmitted helminths, including <i>Strongyloides stercoralis</i>, among children in Zanzibar	147
13.1. Abstract	148
13.2. Introduction.....	149
13.3. Materials and methods	150
13.3.1. <i>Study area</i>	150
13.3.2. <i>Study population</i>	151
13.3.3. <i>Field and laboratory procedures</i>	151
13.3.4. <i>Data management and analysis</i>	152
13.3.5. <i>Ethical considerations and treatment</i>	152
13.4. Results	153
13.4.1. <i>Study compliance</i>	153
13.4.2. <i>Helminth infections</i>	153
13.4.3. <i>Geological features and soil types</i>	155
13.5. Discussion.....	156

13.6. Acknowledgements	159
13.7. References	161
14. Patterns and risk factors of helminth infections and anemia in a rural and a peri-urban community in Zanzibar, in the context of helminth control programs	165
14.1. Abstract	166
14.2. Author Summary.....	167
14.3. Introduction.....	168
14.4. Materials and methods	169
14.4.1. Ethics statement.....	169
14.4.2. Study setting	170
14.4.3. Study participants.....	170
14.4.4. Field procedures.....	171
14.4.5. Laboratory procedures	171
14.4.6. Questionnaire survey.....	173
14.4.7. Data management and statistical analysis	173
14.5. Results	175
14.5.1. Study cohort and compliance	175
14.5.2. Population profile.....	176
14.5.3. Helminth infections and anemia, stratified by study setting	178
14.5.4. Helminth infections and anemia, stratified by age group.....	178
14.5.5. Seroprevalence of helminth infections.....	182
14.5.6. Risk factors for helminth infections and anemia, stratified by study setting	182
14.5.7. Association between helminth infections and anemia, stratified by study setting	185
14.5.8. Association between helminth infections or anemia and self-reported morbidity signs.....	186
14.6. Discussion.....	187
14.7. Acknowledgments.....	192
14.8. References	193
14.9. Translation of abstract into German.....	200
15. Changing patterns of soil-transmitted helminthiases in Zanzibar in the context of national helminth control programs	201
15.1. Abstract	202

15.2.	Introduction.....	203
15.3.	Materials and methods	205
15.3.1.	<i>Study area and population</i>	205
15.3.2.	<i>Field and laboratory procedures</i>	205
15.3.3.	<i>Statistical analysis</i>	206
15.3.4.	<i>Ethical considerations and treatment</i>	207
15.4.	Results	208
15.4.1.	<i>Study profile and compliance</i>	208
15.4.2.	<i>Prevalence and intensity of helminth infections</i>	209
15.4.3.	<i>Multiple helminth infections</i>	211
15.4.4.	<i>Comparison with data obtained in 1994</i>	211
15.4.5.	<i>Results from univariate and multivariate analyses</i>	213
15.5.	Discussion.....	215
15.6.	Acknowledgements	218
15.7.	References	219
16.	Albendazole and mebendazole administered alone or in combination with ivermectin against <i>Trichuris trichiura</i>: a randomized controlled trial	225
16.1.	Abstract	226
16.2.	Introduction.....	227
16.3.	Patients and methods.....	228
16.3.1.	<i>Study area and population</i>	228
16.3.2.	<i>Ethical considerations</i>	228
16.3.3.	<i>Eligibility criteria for participants</i>	228
16.3.4.	<i>Sample size</i>	228
16.3.5.	<i>Baseline parasitological survey</i>	229
16.3.6.	<i>Randomization</i>	229
16.3.7.	<i>Assessment of adverse events</i>	230
16.3.8.	<i>Follow-up</i>	230
16.3.9.	<i>Laboratory procedures</i>	230
16.3.10.	<i>Primary and secondary outcomes</i>	231
16.3.11.	<i>Statistical analysis</i>	231
16.4.	Results	232
16.4.1.	<i>Study Cohort</i>	232
16.4.2.	<i>Baseline Parasitological Survey</i>	232

16.4.3.	<i>Baseline Characteristics</i>	232
16.4.4.	<i>Time difference for follow-up</i>	235
16.4.5.	<i>CR and ERR against T. trichiura</i>	235
16.4.6.	<i>Treatment outcome on hookworm and A.lumbricoides</i>	237
16.4.7.	<i>Adverse Events</i>	238
16.5.	Discussion.....	241
16.6.	Acknowledgments.....	243
16.7.	References	244
17.	Discussion	247
17.1.	The epidemiology of soil-transmitted helminth infections on Unguja	249
17.2.	Helminth control in Zanzibar.....	252
17.2.1.	<i>Success of soil-transmitted helminth control programmes and improved sanitation in Zanzibar</i>	252
17.2.2.	<i>Drug efficacy and safety in Zanzibar and at broader levels</i>	253
17.2.3.	<i>The need for advanced health education and improved environmental sanitation on Unguja and additional suggestions for a more effective soil-transmitted helminth control</i>	256
17.3.	Soil-transmitted helminth diagnostics in the era of preventive chemotherapy	258
17.4.	Can lessons learned from Zanzibar be extrapolated to other helminth control programmes?	261
17.5.	Conclusion.....	265
17.6.	Research needs and recommendations.....	267
17.6.1.	<i>Identified research needs</i>	267
17.6.2.	<i>Recommendations for helminth control in Zanzibar</i>	269
17.7.	References	271
18.	Curriculum vitae	279
19.	Publications	282

Figure index

Figure 1. Global distribution of soil-transmitted helminth infections in 2008..	33
Figure 2. Transmission cycle of soil-transmitted helminth infections.	35
Figure 3. Health education poster to prevent soil-transmitted helminth infections.	41
Figure 4. Improved sanitation coverage, 2006.....	42
Figure 5. Proportion of urban and rural populations practising open defecation, by region in 2006.	42
Figure 6. Map of Unguja, Zanzibar, and its location in Tanzania, Africa.....	62
Figure 7. Flow chart detailing the study participation and compliance of randomly selected children from Chaani and Kinyasini schools, Zanzibar.....	71
Figure 8. Diagrams detailing the differences in the observed and estimated ‘true’ prevalence of soil-transmitted helminth infections employing different diagnostic methods in relation to the number of stool samples from children from Chaani and Kinyasini schools, Zanzibar.	73
Figure 9. Diagram detailing study participation and stool sample submission compliance of randomly selected children attending Chaani and Kinyasini schools, Zanzibar in mid-2007.	93
Figure 10. Prevalence of soil-transmitted helminth infections in children from Chaani and Kinyasini schools, Zanzibar, in mid-2007.	95
Figure 11. Number of stool samples examined with the Kato-Katz and FLOTAC method at baseline and follow-up.....	125
Figure 12. Map showing the geological zones of Zanzibar (adapted from Kent et al., 1971; A) and a satellite image of Zanzibar from Google™-Earth (B) indicating the surveyed settings.	156
Figure 13. Study participation and compliance. Diagram detailing the study participation and compliance of community members from rural Bandamaji and peri-urban Dole, Zanzibar, in June/July 2008.....	176
Figure 14. Prevalence of soil-transmitted helminths, <i>S. haematobium</i> and anemia in rural Bandamaji and peri-urban Dole, Zanzibar, in June/July 2008.....	179
Figure 15. Polyparasitism in rural Bandamaji and peri-urban Dole, Zanzibar, in June/July 2008.	180
Figure 16. Age group specific differences in helminth infection intensities as expressed by egg excretion. Eggs per gram (EPG) values of infected individuals in different age groups of two Zanzibari communities: rural Bandamaji and peri-urban Dole.....	181

Figure 17. Diagram detailing the treatment with mebendazole (dotted arrowed line), albendazole (arrowed line), ivermectin (dashed arrowed line) and praziquantel (dotted and dashed arrowed line) since the onset of large-scale anthelmintic drug administration in 1995 until the cross-sectional survey reported here in June 2007.....	204
Figure 18. Diagram detailing the study participation and compliance of randomly selected school children from Kinyasini and Chaani, Unguja, Zanzibar.....	208
Figure 19. Flow diagram of the randomized controlled trial comparing the efficacy and safety of albendazole and mebendazole alone and in combination with ivermectin against <i>T. trichiura</i> in children from primary schools in Kinyasini and Kilombero on Unguja Island, Zanzibar, in early 2009.....	233
Figure 20. Independent treatment effects of albendazole vs. mebendazole and ivermectin vs. placebo on infection intensities of <i>T. trichiura</i> (administration of albendazole and mebendazole alone and in combination with ivermectin in a 2×2 factorial design).....	238
Figure 21. Percentage of the population of the 6 districts on Unguja with no toilet facilities and the prevalence of soil-transmitted helminth infections in one madrassa of district North A, B, Central, West, and South, and in 5 primary schools in the urban district (red bar: <i>T. trichiura</i> ; blue bar: hookworm; orange bar: <i>A. lumbricoides</i> ; green bar: <i>S. stercoralis</i>).	251
Figure 22. Nexus of soil-transmitted helminth control.....	263

Table index

Table 1. Classes of infection intensity for each soil-transmitted helminth species proposed by WHO (Montresor et al., 1998).	38
Table 2. Characteristics of soil-transmitted helminth infections among children from Chaani and Kinyasini schools, Zanzibar, as determined by the Kato-Katz technique.....	75
Table 3. Sensitivity of individual and combined diagnostic methods if 1 or 3 stool samples from children from Chaani and Kinyasini schools, Zanzibar, were examined (all values expressed as percentage, %) and samples needed to obtain $\leq 1\%$ false negative test results.	77
Table 4. Sensitivity and negative predictive value (NPV) of the first and all three Kato-Katz thick smears, and single FLOTAC examinations for the diagnosis of soil-transmitted helminths among 279 schoolchildren from Zanzibar.....	97
Table 5. Two-way contingency tables showing the agreement between triplicate Kato-Katz thick smears and a single FLOTAC examination (both flotation solutions) for the diagnosis of soil-transmitted helminths in stool samples from 279 schoolchildren from Zanzibar.	98
Table 6. EPG values (expressed as arithmetic mean [AM], standard error [SE], percentiles and maximum) and their statistically significant differences as revealed by a single FLOTAC (two different flotation solutions used, designated S4 and S13) and triplicate Kato-Katz thick smears for the diagnosis of soil-transmitted helminths in stool samples from schoolchildren from Zanzibar (n = 279).	99
Table 7. Arithmetic mean eggs per gram of faeces (EPG), prevalence and sensitivity (including 95% confidence intervals (CI)) of a single FLOTAC and multiple Kato-Katz thick smears as determined in cross-sectional studies with schoolchildren from Côte d'Ivoire and Zanzibar.	111
Table 8. Advantages and disadvantages of the Kato-Katz and FLOTAC techniques for diagnosis of soil-transmitted helminth infections.	112
Table 9. Diagnostic accuracy of duplicate Kato-Katz thick smears and the FLOTAC basic technique at baseline.....	128
Table 10. Diagnostic accuracy of duplicate Kato-Katz thick smears and the FLOTAC basic technique at follow-up.	130
Table 11. Drug efficacy as determined with the Kato-Katz method and FLOTAC basic technique.	132
Table 12. Infection characteristics in 66 untreated children at baseline and follow-up.	134

Table 13. Prevalence (number of children positive/number of children examined) of soil-transmitted helminth infections in 336 children from the 6 districts of Zanzibar, Tanzania.	154
Table 14. Characteristics of the three common soil-transmitted helminth infections among the 327 school-aged children from Unguja who had one stool sample quantitatively examined by the Kato-Katz method.....	155
Table 15. Population characteristics, according to questionnaire survey, stratified by study setting (rural Bandamaji: n = 236, peri-urban Dole: n = 139) in Zanzibar, June/July 2008.	177
Table 16. Risk factors significantly ($p < 0.05$) associated with helminth infections and anemia in individuals from rural Bandamaji and peri-urban Dole in Zanzibar, in June/July 2008, as determined with multivariate logistic regression modeling.....	184
Table 17. Significant associations ($p < 0.05$) between different helminth infections and anemia in residents from rural Bandamaji and peri-urban Dole in Zanzibar, in June/July 2008, as determined with multivariate logistic regression modeling.....	186
Table 18. Self-reported morbidity signs significantly ($p < 0.05$) associated with helminth infections and anemia among residents from rural Bandamaji and peri-urban Dole in Zanzibar, in June/July 2008, as determined with multivariate logistic regression modeling.	187
Table 19. Observed and estimated 'true' prevalence of helminth infections among school children in Chaani and Kinyasini in Unguja, Zanzibar in June/July 2007, and sensitivity of diagnostic methods/method combinations in relation to sampling effort.	210
Table 20. Characteristics of helminth infections in children from Chaani and Kinyasini in Unguja, Zanzibar, as determined with the Kato-Katz method (<i>A. lumbricoides</i> , hookworm and <i>T. trichiura</i>) and the Baermann technique (<i>S. stercoralis</i>) in 1994 and 2007.....	212
Table 21. Association of exposure and infection with soil-transmitted helminths in 362 school children from Chaani and Kinyasini, Unguja, Zanzibar.....	214
Table 22. Baseline demographic and clinical characteristics of 610 children included in the randomized controlled trial conducted in early 2009 in the primary schools of Kilombero and Kinyasini on Unguja Island, Zanzibar, Tanzania.	234
Table 23. Cure rate (CR) and egg reduction rate (ERR) of <i>T. trichiura</i> and infection characteristics of <i>A. lumbricoides</i> and hookworm following administration of albendazole and mebendazole given alone or in combination with ivermectin among 548 children	

from the primary schools of Kilombero and Kinyasini on Unguja Island, Zanzibar, Tanzania.....	236
Table 24. Multiple regression analysis for the 2 x 2 factorial design to assess the risk of a persistent <i>T. trichiura</i> infection after treatment with (i) albendazole or mebendazole and (ii) ivermectin or placebo in 548 school children from the primary schools of Kilombero and Kinyasini on Unguja Island, Zanzibar, Tanzania.	237
Table 25. Adverse events (AEs) reported 48 h after treatment with albendazole or mebendazole in combination with ivermectin or placebo by school children from Kinyasini and Kilombero on Unguja island, Zanzibar (n=564).....	240
Table 26. Contribution of the different chapters of this PhD thesis to the nexus of the Swiss TPH built around the triangle of innovation, validation and application. STH: soil-transmitted helminth.....	248

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2. Summary

Background: Soil-transmitted helminths are intestinal parasitic worms that disproportionately affect socio-economically deprived populations in tropical and subtropical countries living under poor hygienic conditions. The intestinal helminths are transmitted by the contamination of soil with human faeces containing the worms' eggs and by subsequent accidental ingestion of the contaminated soil with food or hands, or by penetration of the worm larvae into the skin and body of humans. The global burden attributed to soil-transmitted helminth infections is estimated to be as high as 39 million disability-adjusted life years (DALYs) lost annually. Children and pregnant women are the groups at highest risk of morbidity. The World Health Organization (WHO) widely advocates periodical deworming of school-aged children as strategy to control morbidity associated with soil-transmitted helminth infections. A global target to reach a minimum treatment coverage of 75% of school-aged children at risk by 2010 was set by the World Health Assembly in May 2001. In Zanzibar, Tanzania, soil-transmitted helminthiasis were recognized as a major public health issue in the early 1990s, with 85% of the surveyed population infected with at least one of the major species, namely *Ascaris lumbricoides*, hookworms (*Ancylostoma duodenale* and *Necator americanus*) and *Trichuris trichiura*. Infections with *Strongyloides stercoralis*, arguably the most neglected soil-transmitted helminth, were found in 40% of schoolchildren examined in rural Zanzibar. In 1994 and 2001 national helminth control programmes were implemented by the Ministry of Health and Social Welfare (MoHSW) of Zanzibar. Since then, anthelmintic drugs have been administered annually to schoolchildren and other eligible population groups of Zanzibar and a treatment coverage of more than 80% was reached.

Goal and specific objectives: The overreaching goal of this PhD thesis was to deepen our understanding of the epidemiology and control of soil-transmitted helminth infections in Zanzibar. There were three specific objectives. First, to compare and evaluate different diagnostic techniques for the detection of soil-transmitted helminth infections. Second, to determine the current epidemiology and risk factors of soil-transmitted helminth infections, including *S. stercoralis*, in environmentally and socio-economically distinct settings on Unguja. Third, to assess the long-term effect of periodic anthelmintic treatment on soil-transmitted helminth prevalences and infection intensities, including the assessment of the efficacy and safety of albendazole and mebendazole administered alone or in combination with ivermectin against *T. trichiura* and other soil-transmitted helminth infections.

Methods: The fieldwork for this PhD thesis was split in three parts. In 2007, for an assessment of the epidemiological situation of helminth infections on Unguja, a cross-sectional study was conducted in five madrassas and five primary schools in the six districts of Unguja and included 336 children. Additionally, a cross-sectional study including 401 children was carried out in Chaani and Kinyasini primary schools. The parasitological results were compared to data derived in 1994 in the same schools. Multiple stool samples were collected from each participant over consecutive days. The Kato-Katz method was employed for the diagnosis of *A. lumbricoides*, hookworm and *T. trichiura*, the Koga-agar plate method for hookworm and *S. stercoralis* and the Baermann method for *S. stercoralis*. Moreover, stool samples preserved in sodium acetate-acetic acid-formalin (SAF) were transferred to Italy and examined with the FLOTAC method. The sensitivity of the individual methods and method combinations was compared.

In 2008, a cross-sectional study was conducted in a rural and a peri-urban setting on Unguja, with 658 individuals aged 5-100 years enrolled. Besides the determination of soil-transmitted helminth infections with the aforementioned methods, urine samples were analysed for *Schistosoma haematobium* infections, blood samples were examined for anaemia and antibodies against helminth infections, and study participants were interviewed with a questionnaire for behavioural risk factors of soil-transmitted helminth infections and associated morbidity signs. The epidemiological situation of both settings was compared and juxtaposed with helminth control activities in Zanzibar.

In 2009, a randomised controlled trial was carried out, comparing monotherapies with combination therapy. During the baseline screening 1240 children from Kinyasini and Kilombero schools were enrolled. The children infected with *T. trichiura* (n=610) were assigned to four treatment arms. For diagnosis, four Kato-Katz thick smears before and 3-5 weeks after treatment were employed and results were compared with outcomes derived by the FLOTAC method.

Results: After several years of helminth control in Zanzibar, the prevalence of soil-transmitted helminth infections on Unguja is still high, ranging between 22% in the urban and 70% in the North A district. Infection intensities are mostly light, and hence the diagnosis based on egg-positivity in stool sample has become a challenge. The sensitivity of the coprological methods can be increased with (i) a rigorous adherence to the bench aids provided by WHO (Kato-Katz method); (ii) the examination of multiple stool samples per individual; (iii) multiple examinations from the same stool sample; and (iv) the combination of diagnostic methods. FLOTAC shows a high sensitivity for the diagnosis of *A. lumbricoides*

(~80%) and *T. trichiura* (~90%). However, the method needs further evaluation and standardization to produce reliable results for hookworm diagnosis. Its potential for application in resource-poor settings and its suitability for drug efficacy assessment needs additional investigation.

On Unguja, soil-transmitted helminthiases are most prevalent in the North A district. Infections with multiple species helminth infections are common, particularly in children from rural areas. Anaemia is prevalent, but was not associated with any soil-transmitted helminth infection in our study. Risk factors for soil-transmitted helminth infections are setting- and species-specific and include, besides demographic factors, the consumption of raw vegetables or salad, no hand-washing after defecation and a recent travel history. *T. trichiura* is the predominant soil-transmitted helminth species on Unguja, with highest prevalences found in Kilombero school (71%). In contrast to hookworm (-77%) and *A. lumbricoides* (-71%), the prevalence of *T. trichiura* was not markedly reduced in the past years (-46%). This phenomenon can be explained by the low efficacies of the drugs albendazole (cure rate (CR): 10%; egg reduction rate (ERR): 40%) and mebendazole (CR: 19%; ERR: 67%) commonly applied in Zanzibar's school-based helminth control programmes. The addition of ivermectin increased the efficacy of particularly mebendazole (CR: 55%; ERR: 97%), but also of albendazole (CR: 38%; ERR: 91%) against *T. trichiura*. Both drugs and drug combinations were highly efficacious against *A. lumbricoides* (ERR: >99%). The treatment outcome of albendazole (CR: 59%; ERR: 94%) was better than that of mebendazole (CR: 35%; ERR: 78%) against hookworm infections, but ivermectin did not improve treatment outcomes. Adverse events were mostly mild and disappeared within 48 hours after treatment, and did not differ between the treatment regimens. The CRs assessed with FLOTAC were lower than with the Kato-Katz method for all three soil-transmitted helminth species.

Conclusion: The Zanzibar helminth control programmes have successfully reduced soil-transmitted helminth prevalences and infection intensities, and hence morbidity. Poverty alleviation accompanied by an increased access of households to improved sanitation has likely reduced the infection rate. Soil-transmitted helminth control on Unguja can now focus on prevalence and transmission control, but will need to revise measures for a sustainable progress. Alternation of albendazole and mebendazole should be considered to more effectively target both hookworm and *T. trichiura*. For improved treatment outcomes against *T. trichiura*, and to also target *S. stercoralis* and ectoparasites, albendazole and mebendazole should be combined with ivermectin whenever people are eligible for this kind of therapy. Since new infections cannot be prevented by anthelmintic drugs alone, and because

T. trichiura, the helminth species that is responsible for the majority of infections on Unguja, is cured ineffectively with the currently available drugs, an increase in hygiene and sanitation is indispensable for sustainable control of soil-transmitted helminths in Zanzibar and elsewhere. A far-reaching dissemination of appealing and plausible health education and communication to school-aged children and communities will be necessary to create the environment for community-led improvements in sanitation (latrine construction, sewage disposal and access to clean water), and to assure their adequate use. Only public consent and the wish for better sanitation at local scale, hand-in-hand with governmental and non-governmental supported poverty alleviation measures can finally result in soil-transmitted helminth elimination in Zanzibar and elsewhere.

3. Zusammenfassung

Hintergrund: Durch Bodenkontakt übertragene parasitische Würmer infizieren vor allem arme Bevölkerungsgruppen in tropischen und subtropischen Ländern, die einen geringen hygienischen Standard haben. Intestinale Würmer werden übertragen indem Fäkalien, die Wurmeier enthalten, in den Boden gelangen und von dort an Nahrung oder Händen klebend in den Mund geraten und geschluckt werden. Bei manchen Wurmarten können auch die in der Erde lebenden Larven in die menschliche Haut eindringen und von dort in den Körper und Darm wandern. Die globale Belastung, die durch die intestinalen Würmer verursacht wird, wird auf 39 Millionen jährlich verlorene *disability adjusted life years* (DALYs) geschätzt. Kinder und schwangere Frauen haben das höchste Erkrankungsrisiko. Die Weltgesundheitsorganisation (WHO) vertritt die Strategie einer breiten und wiederholten Entwurmung von Schulkindern zur Bekämpfung der von intestinalen Wurminfektionen verursachten Erkrankungen. Die Weltgesundheitsversammlung setzte im Mai 2001 zum Ziel, dass eine flächendeckende Entwurmung von mindestens 75% aller Schulkinder, die ein Infektionsrisiko haben, bis im Jahr 2010 erreicht werden soll. In Sansibar, Tansania, wurden durch Bodenkontakt übertragene intestinale Würmer bereits in den 90er Jahren als Gesundheitsproblem erkannt, weil 85% der untersuchten Bevölkerung mit mindestens einer Art, *Ascaris lumbricoides*, Hakenwurm (*Ancylostoma duodenale* und *Necator americanus*) und *Trichuris trichiura*, infiziert war. Infektionen mit *Strongyloides stercoralis*, der am wenigsten Beachtung findenden Art der intestinalen Würmer, traten bei 40% der untersuchten Schulkinder aus dem ländlichen Sansibar auf. In Sansibar wurden 1994 und 2001 nationale Wurmkontrollprogramme vom Gesundheitsministerium etabliert. Seither werden Schulkindern und andere Risikogruppen jährlich entwurmt. Dabei wurde eine Behandlungsdichte von 80% erreicht.

Ziel: Das allumfassende Ziel dieser Dissertation war die Vertiefung unseres Verständnisses der Epidemiologie und Kontrolle von durch Bodenkontakt übertragene Wurminfektionen in Sansibar. Die drei folgenden bestimmten Ziele sollten untersucht werden. Erstens sollten unterschiedliche diagnostische Methoden für die Diagnose von intestinalen Wurminfektionen verglichen und evaluiert werden. Zweitens sollten die Epidemiologie und Risikofaktoren für durch Bodenkontakt übertragene Würmer, einschließlich *S. stercoralis*, in Gebieten von Unguja, Sansibar, die sich in ihrer Umwelt und ihrem Sozioökonomischen Status unterscheiden, untersucht werden. Drittens sollte die Langzeitwirkung von Antiwurm-Behandlungen auf das Vorkommen und die Intensität der Wurminfektionen festgestellt

werden. Hierbei sollte auch die Wirkkraft und Sicherheit von Albendazole und Mebendazole, einzeln oder in Kombination mit Ivermectin gegen *T. trichiura* und andere Würmer verabreicht, ermittelt werden.

Methoden: Die Feldarbeit für diese Dissertation gliederte sich in drei Teile. Im Jahr 2007 wurde eine Querschnittsstudie in fünf Koranschulen und in fünf Grundschulen in den sechs Distrikten von Unguja durchgeführt und 336 Kinder untersucht. Diese Studie diente der Untersuchung der epidemiologischen Begebenheiten von Wurminfektionen in Sansibar. Ebenfalls in diesem Jahr wurde eine Querschnittsstudie mit 401 Kindern aus den Grundschulen in Chaani und Kinyasini durchgeführt. Die parasitologischen Ergebnisse wurden mit Daten aus dem Jahr 1994 verglichen, die in den gleichen Schulen erhoben worden waren. Es wurden mehrere Stuhlproben über mehrere Tage hinweg von jedem der Kinder gesammelt. Die Kato-Katz Methode wurde zur Diagnose von *A. lumbricoides*, Hakenwurm und *T. trichiura* benutzt. Die Koga Agarplatten Methode diente zur Auffindung von Hakenwürmern und *S. stercoralis* und die Baermann Methode zur Diagnose von *S. stercoralis* Infektionen. Zusätzlich wurden Stuhlproben in einer Natriumazetat-Essigsäure-Formalinlösung (SAF) konserviert und in Italien mit der FLOTAC Methode untersucht. Die Sensitivität der einzelnen Methoden und Methodenkombinationen wurde verglichen.

Im Jahr 2008 wurde eine Querschnittsstudie in einer ländlichen und einer vorstädtischen Gemeinde mit 658 Personen im Alter von 5-100 Jahren durchgeführt. Neben der Diagnose von intestinalen Würmern mit den dargelegten Methoden wurden auch Urinproben auf *Schistosoma haematobium* Infektionen untersucht. Blutproben wurden auf Blutarmut und Antikörper gegen Wurminfektionen analysiert und die Teilnehmer wurden in einem Fragebogen-Interview über Verhaltensrisiken und Erkrankungen, die in Zusammenhang mit intestinalen Wurminfektionen stehen könnten, befragt. Die epidemiologische Situation in beiden Gebieten wurde verglichen und in Kontext mit den Wurmkontrollaktivitäten in Sansibar gesetzt.

Im Jahr 2009 wurde eine randomisierte kontrollierte Studie durchgeführt um die Wirkung von Monotherapien mit der von Kombinationstherapien zu vergleichen. Im Rahmen der Studie wurden 1240 Kinder aus den Grundschulen von Kinyasini und Kilombero auf Wurminfektionen untersucht. Die Kinder, die mit *T. trichiura* infiziert waren (n=610), wurden vier Behandlungsgruppen zugeteilt. Zur Diagnose wurden vier Kato-Katz Untersuchungen vor und 3-5 Wochen nach der Behandlung durchgeführt. Die Ergebnisse wurden mit denen der FLOTAC Methode verglichen.

Ergebnisse: Nach mehreren Jahren Wurmkontrollaktivitäten in Sansibar sind immer noch viele Menschen in Unguja mit durch Erdkontakt übertragenen intestinalen Würmern infiziert. Die Prävalenz liegt zwischen 22% im urbanen Distrikt und 70% im Nord A Distrikt. Da die Intensität der Infektionen aber bei den meisten Menschen niedrig ist, sind die Infektionen schwierig zu diagnostizieren. Die Sensitivität der koprologischen Methoden kann (i) durch eine strikte Befolgung der WHO Laborhilfen (Bench aids für die Kato-Katz Methode), (ii) durch die Untersuchung mehrerer Stuhlproben pro Person, (iii) durch mehrere Untersuchungen einer Stuhlprobe pro Person und (iv) durch die Kombination von verschiedenen Methoden verbessert werden. Die FLOTAC Methode diagnostiziert *A. lumbricoides* (~80%) und *T. trichiura* (~90%) Infektionen mit einer hohen Sensitivität. Die Methode muss aber noch weiter evaluiert und standardisiert werden, um Hakenwürmer verlässlich zu diagnostizieren. Das Potenzial der Methode auch in Entwicklungsländern angewendet zu werden und ihre Brauchbarkeit für Medikamentenstudien muss noch weiter untersucht werden. In Unguja kommen intestinale Wurminfektionen am häufigsten im Nord A Distrikt vor. Infektionen mit mehreren Wurmarten auf einmal treten besonders häufig in Kindern aus ländlichen Gegenden auf. Blutarmut kommt ebenfalls häufig vor, war aber in unserer Studie nicht mit intestinalen Wurminfektionen verbunden. Die Risikofaktoren für Wurminfektionen sind von dem Untersuchungsgebiet und der Wurmart abhängig und schließen neben demographischen Faktoren auch den Verzehr von rohem Gemüse oder Salat, das Nicht-Händewaschen nach dem Stuhlgang und eine vorangegangene Reise ein. *T. trichiura* ist die dominierende Art in Unguja und die höchsten Prävalenzen wurden in der Kilombero Grundschule gefunden (71%). Im Gegensatz zu Hakenwurm (-77%) und *A. lumbricoides* (-71%) ging die Prävalenz von *T. trichiura* (-48%) in den vergangenen Jahren weniger stark zurück. Die Ursache hierfür liegt wahrscheinlich in der schlechten Wirkungskraft von Albendazol (Heilungsrate (CR): 10%; Eireduktionsrate (ERR): 40%) und Mebendazol (CR: 19%; ERR: 67%), den Medikamenten, die normalerweise in Schulentwurmungsprogrammen gegen *T. trichiura* und andere intestinale Wurminfektionen verwendet werden. Der Zusatz von Ivermectin konnte die Wirkungskraft von vor allem Mebendazol (CR: 55%; ERR: 97%), aber auch von Albendazol (CR: 38%; ERR: 91%) gegen *T. trichiura* verbessern. Alle Medikamente und deren Kombinationen waren sehr wirkungsvoll gegen *A. lumbricoides* (ERR: >99%). Die Wirkungskraft von Albendazol (CR: 59%; ERR: 94%) gegen Hakenwürmer war sehr viel besser als die von Mebendazol (CR: 35%; ERR: 78%), aber der Zusatz von Ivermectin verbesserte den Behandlungserfolg nicht. Alle Nebenwirkungen waren mild und gingen innerhalb von 48 Stunden zurück und es gab

keinen Unterschied zwischen den vier Behandlungsschemen. Die Heilungsraten, die mit FLOTAC gemessen wurden, waren für alle drei Wurmartentypen niedriger als die Ergebnisse, die mit der Kato-Katz Methode erhalten wurden.

Schlussfolgerung: Die Wurmkontrollprogramme in Sansibar haben die Prävalenz und Intensitäten von durch Erdkontakt übertragenen intestinalen Würmern und die damit in Zusammenhang stehenden Erkrankungen erfolgreich verringert. Eine geringere Armutsrate und ein damit einhergehender verbesserter Zugang zu sanitären Einrichtungen der Haushalte haben sicherlich ebenfalls dazu beigetragen, die Infektionsrate zu verringern. Die Kontrollprogramme von durch Erdkontakt übertragenen intestinalen Wurminfektionen in Unguja können nun die Prävalenz- und Übertragungseinschränkung in Angriff nehmen, müssen aber für einen nachhaltigen Fortschritt einige Massnahmen überarbeiten. Die abwechselnde Anwendung von Albendazol und Mebendazol sollte in Erwägung gezogen werden, um sowohl Hakenwurm als auch *T. trichiura* Infektionen effektiver einzudämmen. Damit *T. trichiura* erfolgreicher behandelt werden kann, und um zusätzlich auch *S. stercoralis* und Ektoparasiten zu erreichen, sollten Albendazol und Mebendazol mit Ivermectin kombiniert werden, wann immer es für Patienten in Frage kommt. Da Neuinfektionen durch Medikamentenanwendung alleine nicht verhindert werden können, und da *T. trichiura* Infektionen, die den Grossteil in Unguja ausmachen, nur ungenügend mit den vorhandenen Medikamenten geheilt werden, ist eine Verbesserung der Hygiene und der sanitären Einrichtungen unabdingbar, um die Wurminfektionen in Sansibar und in anderen endemischen Gebieten der Erde weiter einzudämmen. Eine ansprechende und einleuchtende Gesundheitserziehung und Kommunikation sollte weithin angewandt werden, um die Einstellung der Bevölkerung zu Verbesserungen im Sanitärwesen (Latrinbau, Abwasserentsorgung und Zugang zu sauberem Wasser) zu fördern und ihre angemessene Nutzung zu gewährleisten. Nur ein verbreiteter Konsens und Wunsch nach besseren sanitären Einrichtungen auf lokaler Ebene, im Einklang mit von Regierungs- und Nichtregierungsorganisationen geförderten Armutsbekämpfungsmassnahmen wird letztendlich die Ausrottung von durch Bodenkontakt übertragenen intestinalen Würmern in Sansibar und in anderen Regionen der Welt ermöglichen.

4. Muhtasari

Utangulizi: Kuna aina ya Minyoo ya tumbo inavyoambukiza kwa njia ya udongo, ambayo huathiri sana jamii ya watu maskini wanaoishi nchi za tropiki (nchi za joto) ambao kiwango chao cha usafi ni duni. Minyoo hii huambukiza kupitia udogo uliochanganyika na kinyesi cha binadamu chenye mayai ya minyoo hao na kisha kwa bahati mbaya mtu akaula udongo huo ukiwa umechanganyikana na chakula au mikono michafu, au lava ya minyoo kupenyeza kwenye ngozi au mwili wa binadamu. Maambukizo ya minyoo hawa yanakadiriwa kusababisha ulemavu wa maisha kwa watu wapatao milion 39 kwa mwaka (DALYs). Watoto na wamama wajawazito ndio waathirika wakuu wa maambukizo ya minyoo hawa. Shirika la Afya Duniani (WHO) linahamasisha kudhibiti mara kwa mara wa minyoo hao kama mkakati wa kudhibiti vifo vinavyohusishwa na minyoo hawa kwa watoto wa shule. Dunia imejiwekea lengo la kuwafikia angalau 75% ya watoto wa shule walio katika hatari ya kuambukizwa minyoo ifikapo mwaka 2010, lengo ambalo liliwekwa na Mkutano wa Afya wa Dunia uliofanyika mwaka 2001. Huko Zanzibar, Tanzania, minyoo ya tumbo ilitambuliwa kama tatizo la afya ya jamii mnamo mwaka 1990, ambapo 85% ya watu walikuwa wameambukizwa na moja ya minyoo mikubwa aina ya *Ascaris lumbricoides*, hookworms (*Ancylostoma duodenale* and *Necator americanus*) au *Trichuris trichiura*. Maambukizi ya *Strongyloides stercoralis*, moja aina ya minyoo iliyosahaulika, ilipatikana kwa 40% ya watoto wa shule waliopimwa vijijini Zanzibar. Mnamo mwaka 1995 na 2001, mradi wa kitaifa wa kudhibiti minyoo ya tumbo ulitekelezwa na Wizara ya Afya na Ustawi wa Jamii ya Zanzibar. Tangu wakati ule, dawa za minyoo ya tumbo zimekuwa zikitolewa kila mwaka kwa watoto wa shule na makundi stahili ya jamii visiwani Zanzibar na tiba hii imeweza kuwafikia 80% ya walengwa.

Malengo: Tasnifu hii ya Shahada ya Udaktai wa Falsafa itafuatilia malengo matatu. Lengo la kwanza, kulinganisha na kutathmini njia mbalimbali za uaguzi zitumikazo kubaini maambukizi ya minyoo. Lengo la pili, kubainisha hali ya sasa ya elimu ya magonjwa ya mlipuko na tabia hatarishi za maambuzi ya minyoo ya tumbo pamoja na *S. stercoralis* katika mazingira na uchumi jamii tofauti na ule wa Unguja, Zanzibar. Lengo la tatu, kutathmini matokeo ya muda mrefu ya utoaji wa mara kwa mara wa dawa za tiba za minyoo ya tumbo kwenye maeneo ambayo minyoo ya tumbo imetapakaa na kiwango na ukubwa wa maambukizi na umadhubuti wa dawa zinazotumika mara kwa mara kwenye mradi wa kuzuia minyoo ya tumbo huko Zanzibar.

Mbinu: Kazi ya ukusanyaji taarifa za tasnifu hii iligawanywa katika sehemu tatu. Mwaka 2007, ili kutathmini hali halisi ya maambukizi ya magonjwa ya mlipuko huko Unguja, utafiti wakilishi ulifanyika kwenye Madrassa tano na shule za msingi tano katika wilaya sita za Unguja na kuhushisha watoto 336. Kwa ziada, utafiti mwingine wakilishi uliohusisha watoto 401 ulifanyika kwenye shule mbili za msingi za Chaani na Kinyasini. Matakeo ya vijidudu vilivyoonekana yalilinganishwa na matokeo ya utafiti ya mwaka 1994 katika shule hizo hizo. Sampuli kadhaa za kinyesi kwa kila mshiriki zilikusanywa kwa siku kadhaa mfululizo. Mbinu ya Kato-Katz ilitumika kutambua vijidudu vya *A. lumbricoides*, tegu na *T. trichiura*, na mbinu ya Koga-agar kwa ajili ya tegu na *S. stercoralis* na pia mbinu ya Baermann kwa ajili ya maambuzi ya *S. stercoralis*. Lakini pia, sampuli za kinyesi zilihifadhiwa kwenye madawa ya Sodium Acetate-acetic acid-formalin (SAF) zilisafirishwa kwenda Italia na kupimwa kwa kipimo cha FLOTAC. Viwango vya hisi kwa mbinu hizi na mbinu zingine mchanganyiko vililinganishwa. Mwaka 2008, utafiti wakilishi ulifanyika maeneo ya vijijini na kando kando ya mji wa Unguja na kuhushisha watu 658 wenye umri wa kati ya miaka 5-100. Mbali na uchunguzi wa minyoo ya tumbo kwa mbinu zilizelezwa hapo juu, sampuli za mkojo zilipimwa kubaini *Schistosoma haematobium*, sampuli za damu zilipimwa kubaini upungufu wa damu na kinga dhidi ya maambukizi ya minyoo ya tumbo, na washiriki wa utafiti walihojiwa kwa dodoso kuhusu tabia hatarishi kwa maambukizi ya minyoo ya tumbo na dalili shiriki ziambatanazo na ugonjwa. Kuenea kwa mlipuko katika maeneo hayo mawili Zanzibar kulilinganishwa. Mwaka 2009, utafiti wakilishi ambao haukuzingatia maeneo maalumu ulifanyika na kuhushisha watoto 1240 kutoka shule za Kinyasini na Kilombero, watoto 610 walioambukizwa na vijidusu vya *T. trichiura* waligawanywa kwenye makundi (mikondo) manne ya tiba. Umadhubuti wa dawa ya albendazole na mebendazole pekee au mchanganyiko wa ivermectin vilitathimiwa kwa kipimo cha kato-katz na matokeo yake kulinganishwa na kipimo cha FLOTAC.

Matokeo: Baada ya miaka mingi ya kudhibiti minyoo ya tumbo huko Zanzibar, ukubwa wa maambukizi ya minyoo ya tumbo bado uko juu, kati ya 22% ya maeneo ya mjini na 70% katika wilaya ya Kazkazini A. Kiwango cha maambukizi hakikuwa kikubwa, na hivyo utambuzi wa minyoo ya tumbo ukawa na changamoto. Kiwango cha hisi cha mbinu za *coprological* kinaweza kuongezeka kwa: i) kuzingatia kwa usahihi msadaa wa kiofisi unaotolewa na Shirika la Afya Duniani (Kato-Katz); ii) kufanya uchunguzi wa sampuli nyingi za kinyesi kwa kila mtu, iii) chunguzi kadhaa kwa sampuli moja ya kinyesi, na iv) mchanganyiko wa mbinu. FLOTAC inaonyesha mwikio mkubwa wa utambuzi wa *A. lumbricoides* (~80%) na *T. trichiura* (~90%). Hata hivyo, mbinu hii inahitaji tathmini na

vigezo zaidi kuweza katoa matokeo ya uhakika kwa utambuzi wa minyoo aina ya tegu. Inafaa kutumika katika maeneo yenye uhaba wa rasilimali lakini usahihi wake kwa tathmini ya uthabiti wa dawa unahitaji utafiti zaidi. Huko Unguja, minyoo ya tumbo wanaoenezwa kwa njia ya udongo wapo kwa wingi sana katika wilaya ya Kaskazini A. Maambukizi ya aina nyingi ya minyoo ya tumbo yapo kwa wingi, hasa kwa watoto wanaotoka maeneo ya vijijini. Upungufu wa damu upo kwa wingi lakini haukuhusishwa na aina yeyote vya minyoo ya tumbo inayoambukizwa kupitia udongo katika utafiti wetu. Visababishi hatarishi vya minyoo ya tumbo inayoambukizwa kupitia udongo yanaendana na mazingira na jamii ya minyoo na inajumuisha, mbali na sababu za kidemografia, matumizi ya mboga za majani zisizoiva au kachumbari, kutonawa nikono baada ya haja kubwa na tabia ya kusafiri safiri iliyojitokeza kabla ya uchunguzi. *T. trichiura* ni jamii ya minyoo ya tumbo ambayo imeenea kwa wingi Unguja, na maambukizi mengi yapo shule ya Kilombero (71%). Tofauti na *A. lumbricoides* (-71%) na tegu (-77%), maambukizi ya *T. trichiura* hayakupungua kwa kiwango kikubwa katika miaka iliyopita (-46%). Hali hii inaweza kusababishwa na uthabiti duni wa dawa aina ya albendazole (kiwango cha kutibia (CR): 10%; kiwango cha kupunguza mayai (ERR): 40%) na mebendazole (CR: 19%; ERR: 67%) ambazo zinatumiwa mara nyingi katika mipango ya kudhibiti minyoo ya tumbo mashuleni huko Zanzibar (*T. trichiura* na minyoo mingine inayoambukizwa kupitia udongo). Kuongezwa kwa ivermectin kumeongeza uthabiti wa mebendazole (CR: 55%; ERR: 97%), lakini pia albendazole (CR: 38%; ERR: 91%) dhidi ya *T. trichiura*. Dawa zote na muunganiko wa dawa zilikuwa thabiti dhidi ya *A. lumbricoides* (ERR: >99%). Matokeo ya tiba ya albendazole (CR: 59%; ERR: 94%) yalikuwa mazuri zaidi kuliko yale ya mebendazole (CR: 35%; ERR: 78%) dhidi ya maambukizo ya tegu, lakini *ivermectin* haikuboresha matokeo ya tiba. Matukio mabaya yalikuwa kidogo sana na yalipotea ndani ya masaa 48 baada ya matibabu, na hii haikutokana na matumizi ya dawa, tofauti miongoni mwa kanuni mbalimbali za tiba zilizotumiwa. Kiwango cha tiba na kupungua kwa mayai kwa tathmini ya FLOTAC kilikuwa chini kuliko tathmini ya Kato-Katz kwa jamii zote tatu za minyoo ya tumbo inayoambukiwa kupitia udongo.

Hitimisho: Mipango ya udhibiti wa minyoo nchini Zanzibar imefanikwa kupunguza viwango vya maambukizi ya minyoo ya tumbo kupitia udongo kwa miaka iliyopita na hivyo athari zinazoambatana na maambukizi hayo zilipungua. Kushuka kwa kiwango cha umaskini uliombatana na kuongezeka kwa matumizi ya vyoo kumechangia hali hii. Udhibiti wa minyoo ya tumbo inayoambukiwa kupitia udongo katika Unguja, unaweza sasa kujikita katika kiwango na udhibiti wa maambukizi, lakini utahitaji kuboresha mikakati yake ili kupata

maendeleo endelevu. Matumizi ya albendazole na mebendazole yanapaswa kubadilishwa kuelekezwa kwa tegu na *T. trichiura*. Kwa ajili ya tiba bora (matokeo bora ya tiba) dhidi ya *T. trichiura*, na pia kulenga *S. stercoralis* na ectoparasites, albendazole na mebendazole lazima ziunganishwe na *ivermectin* kwa wale wanaostahili tiba ya aina hii. Kwa kuwa maambukizi mapya hayawezi kuzuiwa na matumizi ya dawa peke yake, na kwa sababu *T. trichiura* ambao ndio chazo cha maambukizi mengi Unguja, hawatibiki kwa uthabiti kwa dawa zilizopo hivi sasa, kuongozeka kwa usafi wa mazingira (ikiwemo mifumo ya maji taka) ni muhimu kwa ajili ya kudhibiti zaidi minyoo ya tumbo nchini Zanzibar. Elimu ya afya kutolewa kwa watoto wa shule na jamii ni lazima ili kuweka mazingira ya maboresho ya usafi wa mazingira (ujenzi wa vyoo, makaro ya maji taka, na upatikanaji wa maji salama), na matumizi yake sahihi. Ni ridhaa ya jamii pekee na utashi wa usafi wa mazingira katika ngazi ya chini, pamoja na hatua za serikali na asasi zisizo za kiserikali kuelekea kupunguza umaskini hatimaye zinaweza kutokomeza kabisa minyoo ya tumbo inayoambukiwa kupitia udongo nchini Zanzibar na kwingineko duniani.

5. Introduction

5.1. Helminth infections

Parasitic worm (helminth) infections remain a major public health problem, particularly in tropical and subtropical regions. Helminths primarily affect socio-economically deprived populations. It is currently estimated that over a third of the world's population is infected with helminths (de Silva et al., 2003; Bethony et al., 2006). This PhD thesis focuses on the soil-transmitted helminths. The most common soil-transmitted helminths are the large roundworm (*Ascaris lumbricoides*), the whipworm (*Trichuris trichiura*), and the hookworms (*Ancylostoma duodenale* and *Necator americanus*). Another common, but with conventional diagnostic methods not detectable and therefore often neglected soil-transmitted helminth, is the dwarf threadworm *Strongyloides stercoralis*. Together, they are estimated to infect more than a billion people globally and are highly prevalent in sub-Saharan Africa (Figure 1) (WHO, 2005; Bethony et al., 2006). Soil-transmitted helminth infections are a major cause of diarrhoea, abdominal pain, general malaise and weakness, and may impair physical and intellectual growth (Jernigan et al., 1994; Bethony et al., 2006). In addition, hookworm infections are associated with anaemia (Hotez et al., 2004). The global burden attributed to soil-transmitted helminthiasis is estimated to be as high as 39 million disability-adjusted life years (DALYs) lost annually (Bethony et al., 2006; Hotez et al., 2006).

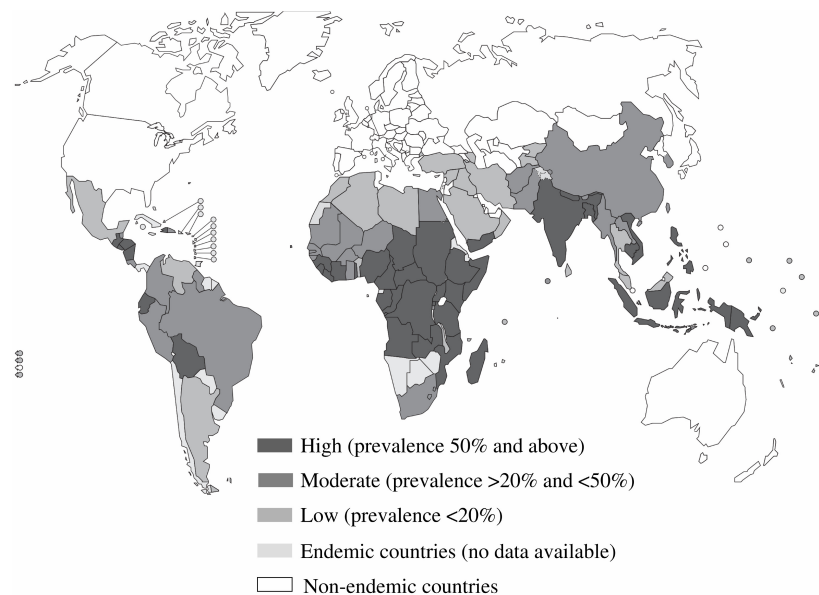


Figure 1. Global distribution of soil-transmitted helminth infections in 2008 (WHO, 2010).

5.1.1. Life cycle and biology of soil-transmitted helminths

Humans are the definitive host of soil-transmitted helminths. These parasitic worms thrive wherever suitable environmental conditions co-exist with inadequate sanitation and poor hygiene (Albonico et al., 2006). Transmission occurs through soil contaminated with faeces. The soil plays an indispensable role in the transmission of these helminth infections. It is necessary for the incubation of *A. lumbricoides* and *T. trichiura* eggs and the free-living larvae of hookworm and *S. stercoralis* to infective stages. As shown in Figure 2, humans become infected through ingestion of parasite eggs or penetration of infectious larvae through the skin (Bethony et al., 2006).

A. lumbricoides and *T. trichiura* are transmitted passively by ingestion of the eggs by the host as a result of faecal contamination (Bradley and Jackson, 2004). In the host *A. lumbricoides* larvae undergo an extensive migration through a series of host tissues and organs, while *T. trichiura* larvae develop entirely in the gut. The adults of both species inhabit the intestinal tract; *A. lumbricoides* parasitizes the entire small intestine, whereas *T. trichiura* lives in the large intestine. Their pre-patent period takes 50-80 days (Utzinger and Keiser, 2004). In the patent period, a female *A. lumbricoides* can produce more than 200,000 eggs per day, whereas *T. trichiura* is producing only around 70 eggs per day (Utzinger and Keiser, 2004). These eggs are excreted with the faeces and can contaminate the soil if people are defecating into the open environment instead of using a latrine.

Hookworm and *S. stercoralis* infections are acquired by invasion of the infective third stage larvae (L3) through the skin. Following host entry, the larvae undergo a journey through the vasculature, enter the airways, are swallowed, and finally reach the intestine, where hookworm larvae moult twice to become adult male and female worms, while *S. stercoralis* larvae mature into egg-laying females (Siddiqui and Berk, 2001; Hotez, 2004). The pre-patent period of a hookworm infection ranges between 28-50 days, depending on the species. Female *N. americanus* produce between 9,000 and 10,000 eggs per day and *A. duodenale* between 25,000 and 30,000 (Bethony et al., 2006). In contrast to the other soil-transmitted helminths, which do not reproduce within the host (Bethony et al., 2006), *S. stercoralis* infections can be perpetuated over long periods by autoinfection (Keiser and Nutman, 2004; Vадlamudi et al., 2006). In that case, larvae develop to the infective L3 within the gastrointestinal tract, penetrate the intestinal mucosa and migrate to the definitive site in the small intestine or to parenteral sites, e.g. the lungs (Grove, 1996; Jorgensen et al., 1996). Additionally, *S. stercoralis* can have a free-living non-parasitic life cycle ending in either infective L3

(homogonic pathway) or free-living adults (heterogonic pathway) determined by environmental and genetic cues (Ashton et al., 1998; Grant and Viney, 2001).

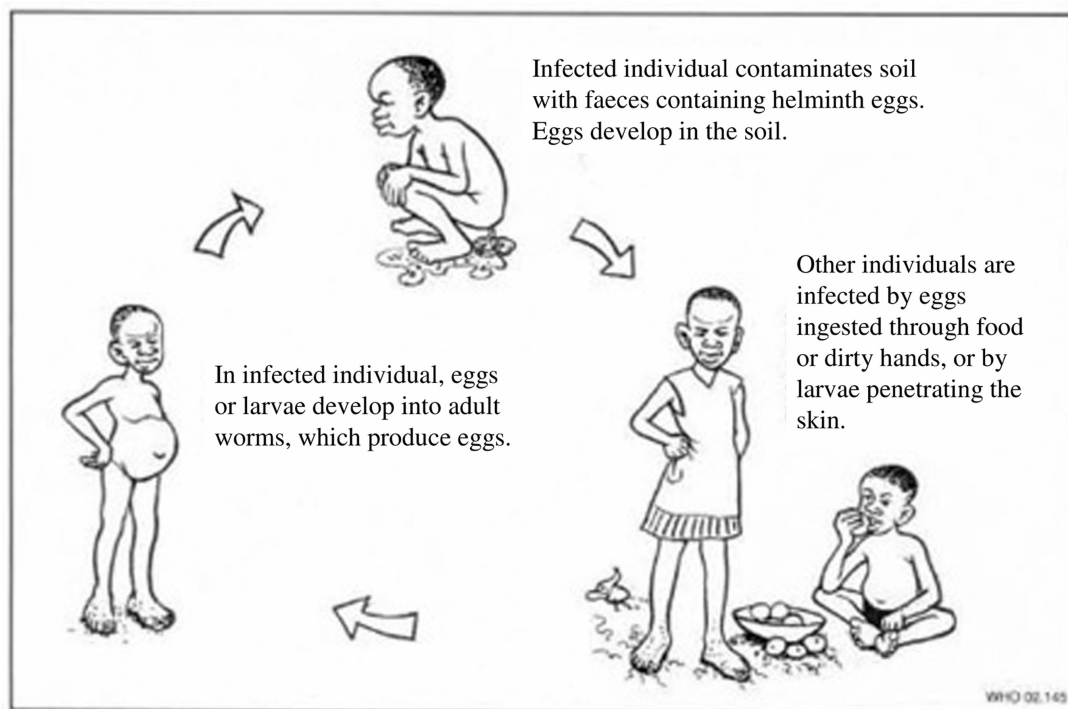


Figure 2. Transmission cycle of soil-transmitted helminth infections (source: <http://www.who.int/wormcontrol/statistics/en/cycle.jpg>; accessed: 26.02.2010).

5.1.2. Morbidity and burden of soil-transmitted helminthiases

Soil-transmitted helminths frequently cause chronic and debilitating diseases, mainly in infants, preschool and school-aged children, adolescent girls and pregnant women (Bethony et al., 2006; Lammie et al., 2006; Goodman et al., 2007). Since soil-transmitted helminth infections cause disability rather than deaths, their global burden is typically expressed in DALYs which is estimated to be as high as 39 million, similar to that owing to malaria or tuberculosis (WHO, 2002b; Hotez et al., 2006). Recent estimates suggest that globally 807-1221 million people are infected with *A. lumbricoides*, 604-795 million with *T. trichiura*, 576-740 million with hookworms and 30-100 million with *S. stercoralis* (de Silva et al., 2003; Bethony et al., 2006). Concurrent infections with multiple helminth species are common (Booth et al., 1998; Keiser et al., 2002; Brooker et al., 2004; Raso et al., 2004).

Morbidity due to soil-transmitted helminth infection and the rate of transmission are related to the number of worms harboured by the host (Anderson, 1991). *A. lumbricoides* is a

well known cause of malnutrition, intestinal obstruction, biliary colic and pancreatitis (Khuroo, 1996). *T. trichiura* infections can induce *Trichuris* dysentery syndrome, whose symptoms include rectal prolapse, anaemia and clubbing of fingers (Bundy and Cooper, 1989). Hookworm is implicated as the causative factor in more than 50% of cases of iron-deficiency anaemia in Asia and Africa (Hotez, 2004).

The pathology of *S. stercoralis*, in common with other infections of nematodes, is often not overt (Genta and Caymmi Gomez, 1989). One aspect of disease might be the compromise of the infected individual's nutritional status (Stephenson et al., 2000). In immuno-suppressed individuals, strongyloidiasis can lead to dangerous disseminated infections with pulmonary haemorrhage, necrotizing colitis and a fatality rate of more than 80% (Igra-Siegman et al., 1981).

Generally, infections with soil-transmitted helminths have a negative impact on pregnancy and birth outcomes, hamper children's cognitive and physical development, result in reduced work capacity, and therefore compromise the social and economic development of communities and entire nations (WHO, 2005; 2006a; Hotez et al., 2006). Infection levels of *A. lumbricoides* and *T. trichiura* are highest in children between the age of 5 and 14 years (WHO, 2002b) and there usually is a decline in frequency and intensity in adulthood (Bundy, 1990). This age dependency might be due to changes in exposure and/or acquired immunity (Galvani, 2005). Hookworm infection in contrast either steadily rises in intensity with age or plateaus in adulthood (Brooker et al., 2004; Hotez, 2004). Reports about the prevalence and infection intensity of *S. stercoralis* in different age groups are rare and conflicting results have been presented from different settings (Prociv and Luke, 1993; Lindo et al., 1995; Dancesco et al., 2005; Steinmann et al., 2007).

5.1.3. Soil-transmitted helminth infections in Zanzibar

Today, more than one quarter of sub-Sahara's population is infected with at least one species of soil-transmitted helminths (de Silva et al., 2003; Hotez and Kamath, 2009) and multiple species infections are the rule rather than the exception (Booth et al., 1998; Lwambo et al., 1999; Raso et al., 2004). Zanzibar, where the focus of this PhD is on, belongs to the United Republic of Tanzania and consists of two major islands, Unguja and Pemba. On both islands soil-transmitted helminth infections were recognized as a major public health problem in the early 1990s (Renganathan et al., 1995; Marti et al., 1996). Indeed 85% of the surveyed population and 99% of examined schoolchildren were infected with at least one of the three

most common soil-transmitted helminths (Renganathan et al., 1995; Marti et al., 1996; Albonico et al., 1997). Point-prevalences of *S. stercoralis* infections were 35% and 41% in schoolchildren examined in Unguja (Marti et al., 1996) and Pemba (Stoltzfus et al., 1997), respectively. Despite helminth control programmes implemented in Zanzibar for more than a decade to combat soil-transmitted helminthiasis, schistosomiasis and lymphatic filariasis (Renganathan et al., 1995; Mohammed et al., 2006; 2008; Rudge et al., 2008), the prevalence of soil-transmitted helminthiasis remains to be relatively high. Recent studies revealed a point-prevalence for soil-transmitted helminth infections of 77% in all age groups in Kinyasini village, Unguja, (Mohammed et al., 2008) and mean-prevalences of 11.3%, 12.5%, and 4.3% of *A. lumbricoides*, *T. trichiura*, and hookworm infections, respectively, in children from 24 schools across Unguja, surveyed in 2006 (Stothard et al., 2009).

5.2. Control of soil-transmitted helminth infections

Periodic deworming with benzimidazoles (i.e. albendazole and mebendazole), complemented by health education, basic sanitation and clean water, is considered the most cost-effective approach to control the morbidity caused by soil-transmitted helminth infections (Savioli et al., 2002; WHO, 2002b; de Silva et al., 2003; Albonico et al., 2006). The ultimate goal of helminth control is to improve health, education, economic status and social well-being of entire populations (WHO, 2006b).

5.2.1. Large-scale deworming

The World Health Organization (WHO) promotes large-scale anthelmintic drug administration as a public health tool to reduce the worm burden and to prevent morbidity over time. The drugs are administered either alone or in combination to at-risk groups without prior diagnosis. This strategy has been termed “preventive chemotherapy” and is considered safe, easy, cheap and effective in reducing the burden due to soil-transmitted helminthiasis (World Bank, 2003; WHO, 2006b). Periodic deworming campaigns lead by the WHO and other organizations have focused on schools, because the heaviest intensities of soil-transmitted helminth infections are most commonly encountered among school-aged children, and schools provide a sound infrastructure for delivering anthelmintic drugs and are in close contact to the whole community (World Bank, 2003). A resolution urging member states to attain a minimum target of regular deworming of at least 75% and up to 100% of all at-risk

school-age children by 2010 was passed at the 54th World Health Assembly in May 2001 (WHO, 2002b). Additionally, preschool children and women of reproductive age are frequently treated in mother and child health programmes (WHO, 2002b). The benefits of regularly administering anthelmintic drugs to school-aged children include improvement of iron stores (Stephenson et al., 1989), growth and physical fitness (Stephenson et al., 1989; Stephenson et al., 1993), and school attendance (Miguel and Kremer, 2003). Women, who are treated once or twice during pregnancy are less anaemic and birth weight and infant mortality at six months are improved (Atukorala et al., 1994; Torlesse and Hodges, 2001; Christian et al., 2004).

However, even if the removal of adult worms from the intestinal tract is the goal of anthelmintic drug administration, the success of large-scale treatment in highly endemic regions is more accurately measured when evaluating infection intensities (Bundy et al., 1992; Bethony et al., 2006). These are usually classified according to thresholds defined by WHO in high, medium and low (Table 1), depending on the number of eggs detected in 1 g of stool (EPG) and are regarded not only as indicator for the worm-burden in the host but also for the degree of environmental contamination with eggs and hence transmission (Montresor et al., 1998). For *S. stercoralis* no thresholds exist, since larvae are not necessarily excreted in stool but infections can be perpetuated by autoinfection (Keiser and Nutman, 2004; Vadlamudi et al., 2006). Generally, the frequency of periodic deworming needs to be adapted to the intensity of transmission and rates of re-infection (Albonico et al., 2006).

	light intensity infections	moderate intensity infections	heavy intensity infections
<i>A. lumbricoides</i>	1-4,999 EPG	5,000-49,999 EPG	> 50,000 EPG
<i>T. trichiura</i>	1- 999 EPG	1,000- 9,999 EPG	> 10,000 EPG
Hookworm	1-1,999 EPG	2,000- 3,999 EPG	> 4,000 EPG
<i>S. stercoralis</i>	not applicable	not applicable	not applicable

Table 1. Classes of infection intensity for each soil-transmitted helminth species proposed by WHO (Montresor et al., 1998).

5.2.2. Anthelmintic drug efficacy

The drugs commonly applied in control programmes targeting soil-transmitted helminth infections are albendazole and mebendazole. They belong to the group of benzimidazoles and lead to the death of adults worms by destroying their tegument by inhibition of microtubule polymerization (Lacey, 1990). However, even if considered to be broad-spectrum

anthelmintics, the two drugs have different species-specific efficacies (Bethony et al., 2006; Keiser and Utzinger, 2008). Albendazole in a single oral dose (usually administered at 400 mg) is highly efficacious against *A. lumbricoides* and hookworm infections, but is less so in curing *T. trichiura* (Olsen, 2007) and *S. stercoralis* infections (Marti et al., 1996). In a recent meta-analysis, considering only randomised placebo controlled trials, the cure rates of albendazole were 88%, 72%, and 28% against *A. lumbricoides*, hookworm and *T. trichiura* infections, respectively (Keiser and Utzinger, 2008). Egg reduction rates of albendazole ranged between 87% and 100%, 64% and 100%, and 0% and 90% for *A. lumbricoides*, hookworm and *T. trichiura* infections, respectively (Keiser and Utzinger, 2008). Mebendazole applied in a single oral dose (usually administered at 500 mg) was reported to be more effective than albendazole against *A. lumbricoides* and *T. trichiura* infections with cure rates of 95% and 36%, and with egg reduction rates of 96-99% and 81-93%, respectively (Keiser and Utzinger, 2008). Its overall cure rate of only 15% and egg reduction rates of 0-93% against hookworm infections are, however, very low (Keiser and Utzinger, 2008). Another drug, ivermectin, is the first-line treatment in programmes to eliminate filarial worms (*Wuchereria bancrofti* and *Onchocerca volvulus*) and is also effective against soil-transmitted helminth infections (Marti et al., 1996). The effect of ivermectin results from paralyzing body wall and pharynx muscles of nematodes (Geary, 2005). Of note, ivermectin is the drug of choice to treat *S. stercoralis* infections, and in contrast to mebendazole and albendazole, it is effective against the dwarf threadworm already in a single oral dose (Gann et al., 1994; Marti et al., 1996). In filariasis control programmes, ivermectin is often combined with albendazole. This combination therapy shows better treatment outcomes against *T. trichiura* infections than the use of albendazole or mebendazole alone, and cure rates of 50-80% and egg reduction rates of 68-98% have been reported (Beach et al., 1999; Belizario et al., 2003; Olsen, 2007). The efficacy of mebendazole plus ivermectin has not yet been assessed.

There is growing concern about a potential reduction in effectiveness or even an emergence of drug-resistance through the widespread and frequent use of anthelmintics (Albonico et al., 2004; Flohr et al., 2007). In areas where large-scale anthelmintic treatment is frequent, the efficacy of the drugs should be monitored and regularly re-assessed to duly recognize treatment failures (Albonico et al., 2006). The combined application of drugs with different mechanisms of action or their alternate use might avoid or delay the onset of drug resistance (Albonico et al., 2006). Combination therapy is recommended against malaria, tuberculosis and HIV/AIDS and many other diseases (Nosten and Brasseur, 2002; WHO, 2003; Hammer et al., 2008), and hence should also be considered in the case of helminthic

diseases (Utzing et al., 2003; Olsen, 2007). The development of new drugs as backup in case of resistance development remains a crucial future perspective for helminth control (Keiser and Utzinger, 2007).

5.2.3. Health education

Intervention outcomes achieved by “preventive chemotherapy” need to be reinforced by good hygienic practices. Poor hygiene and poor sanitation are not synonymous with poverty and by simple changes in lifestyles through education, major changes can also be achieved in resource poor communities (Smits, 2009). However, latrine and safe water use can only be effectively advocated if people are aware of the impact of sanitation on their health and understand transmission cycles of soil-transmitted helminths and other pathogenic agents. In addition to direct positive impacts on health, appropriate health education might optimize treatment coverage, and should be delivered in the early stage of helminth control programmes (Smits, 2009).

The key messages that need to be understood and converted into practice by communities to reduce soil-transmitted helminth transmission are the following: (i) worm infections are negatively affecting health; (ii) since worm eggs are passed out in faeces good latrine habits are vital to prevent and control worm transmission; (iii) one unhygienic or careless individual can infect many other people; (iv) a good personal hygiene, including washing of hands with soap after defecation and farming and before eating is important to avoid an infection with soil-transmitted helminths; (v) vegetables or fruits need to be washed, peeled or cooked before consumption to remove or kill helminth eggs; (vi) the risk of a hookworm infection can be reduced by wearing shoes and by stopping young children playing near places where stools are passed. These habits may be best achieved and sustained if they are associated with increased availability of clean water for hygiene purposes, access to improved sanitation and other poverty alleviation measures (Albonico et al., 2006; Prüss-Üstün et al., 2008).

Health education can be given from trained health workers on deworming days to the community, or be delivered by teachers in form of lessons to schoolchildren that disseminate their gained knowledge to parents and siblings. Behavioural instructions can also be promoted on posters (Figure 3) or in health education booklets in a child-friendly format. Health education as one-directional health instruction might, however, not be very efficient and long-lasting. Behavioural change and action against adverse conditions favouring disease transmission can only be achieved with sound and interactive health communication in the

community, which creates a critical perception of the social, cultural, political and economic forces that structure reality (Porter et al., 1999).



Figure 3. Health education poster to prevent soil-transmitted helminth infections (source: http://www.who.int/wormcontrol/education_materials/tanzania/en/swahili_sth_poster.pdf; accessed: 02.03.2010)

5.2.4. Provision of adequate sanitation and clean water

“Sanitation in the context of economic development is the only definitive intervention that eliminates soil-transmitted helminth infections” (Albonico et al., 2006). However, the sanitary infrastructure is poor in many parts of the world, especially in Asia and sub-Saharan Africa. Worldwide 2.5 billion people lack access to adequate sanitation and 884 million people live without access to an improved water source (Prüss-Üstün et al., 2008; WHO, 2008). As indicated in Figure 4, sub-Saharan Africa is the region with the lowest coverage of “improved” sanitation (31%) in 2006 (WHO, 2008). Improved sanitation facilities ensure hygienic separation of human excreta from human contact and include (i) flush toilets or latrines connected to a piped sewer system, septic tank or pit latrine; (ii) ventilated improved pit latrines; (iii) pit latrines with slab; or (iv) composting toilets. Unimproved toilet facilities

that do not ensure hygienic separation of human excreta from human contact were used by 23% of sub-Saharan's population. Unimproved facilities include pit latrines without a slab or platform, hanging latrines and bucket latrines.

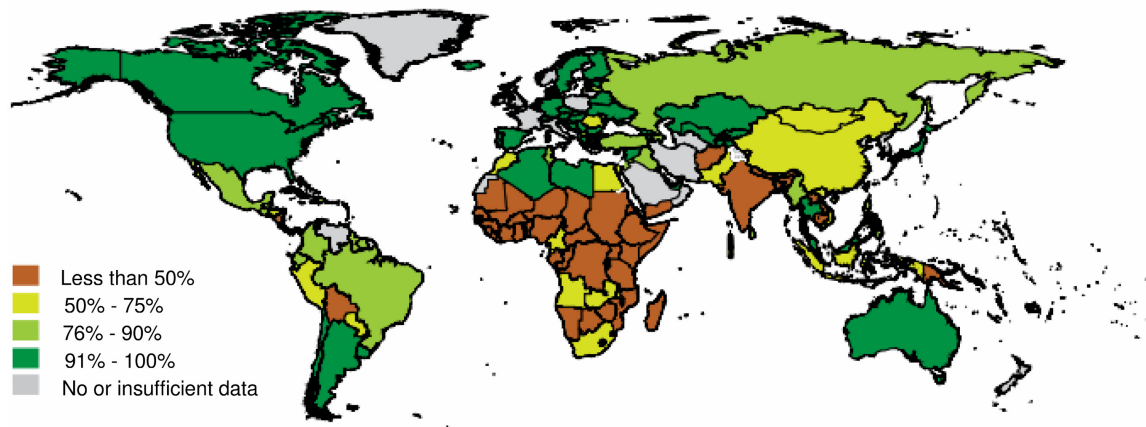


Figure 4. Improved sanitation coverage, 2006 (source: WHO, 2008).

Toilet facilities were shared between several households by 18%. Open defecation, defined as defecation in fields, forests, bushes, bodies of water or other open spaces, or disposal of human faeces as solid waste was practiced by 28% of sub-Saharan's population, particularly in rural areas (Figure 5) (WHO, 2008). Improved drinking water was accessible for 64% of all Africans in 2006 (WHO/UNICEF, 2008). Improved drinking water sources include piped water into dwellings, plots or yards, public taps or standpipes, tubewells or boreholes, protected dug wells, protected springs and rainwater.

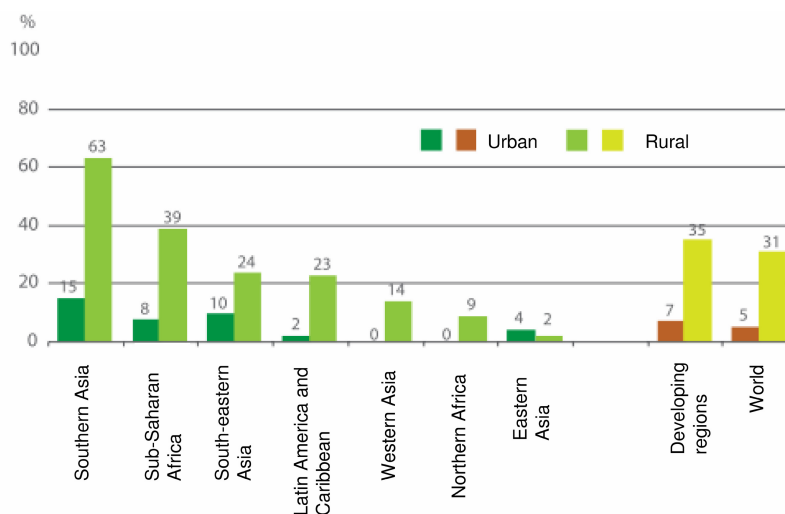


Figure 5. Proportion of urban and rural populations practising open defecation, by region in 2006 (source: WHO, 2008).

The coverage of properly built, used and maintained sanitation must be higher than 90% to become effective (Esrey et al., 1991; Asaolu and Ofoezie, 2003). The financial resources to improve sanitary infrastructures are, however, enormous and will be difficult to overcome in socio-economically deprived communities. A promising and relatively cheap approach to reduce faecal environmental contamination is “community-led total sanitation” (CLTS) (Kar and Chambers, 2008; Chambers, 2009). The goal of CLTS is to initiate local action of communities to look for their own alternatives to open defecation by facilitating the community’s analysis of their sanitation profile, their practices of defecation and the consequences. CLTS processes can lead to improvement of latrine design, the adoption and improvement of hygienic practices; solid waste management, waste water disposal, protection and maintenance of drinking water sources and other environmental measures. So far, CLTS has been successfully introduced in more than 15 countries in Asia, Africa, Latin America and the Middle East (Kar and Chambers, 2008).

5.2.5. Helminth control programmes in Zanzibar

Zanzibar is an example for long-term chemotherapy-based helminth control programmes with both remarkable success and persistent problems. Studies carried out in Pemba and Unguja in the late 1980s and early 1990s revealed that the prevalence of helminth infections exceeded 85% in all surveyed population groups with a considerable amount of moderate, but only few heavy infection intensities (Renganathan et al., 1995; Marti et al., 1996). Therefore, in 1992, a national plan of action for the control of helminths was implemented by the Ministry of Health and Social Welfare (MoHSW) (Renganathan et al., 1995). A target was set to reduce the intensity of infection by 60% for *A. lumbricoides*, 30% for hookworms and 20% for *T. trichiura* in schoolchildren within two years. To achieve this goal, periodic mass-treatment of schoolchildren with a single oral dose of mebendazole (500 mg) was adopted as the most cost-effective strategy for the control of morbidity (Renganathan et al., 1995). As re-infection intensities were found to reach pre-treatment levels already 6 months after treatment, it was decided to treat the children every four to six months (Albonico et al., 1995; Renganathan et al., 1995). Finally, in 1994, the MoHSW established a national helminth control programme (Albonico et al., 1999). Since then, large-scale preventive chemotherapy interventions are regularly conducted at schools and occasionally in whole communities (Mohammed et al., 2008). Currently, on Unguja, the helminth control programme operates from the Helminth Control Laboratory Unguja (HCLU), which provides, when sufficient donor funds are

available, the necessary infrastructure and resources for mass drug administration campaigns targeted mainly towards school-aged children (Stothard et al., 2008). Within the *Piga vita Kichocho* programme (Kick-out schistosomiasis) implemented from 2003-2006 on Unguja, around 14,000 school-aged children were treated annually with albendazole (400 mg) to combat soil-transmitted helminthiasis and praziquantel (40 mg/kg) against infections with *Schistosoma haematobium* (Stothard et al., 2009). In addition, the whole population of Unguja, with the exception of those who were severely sick, children below the age of 5 years, and of pregnant women and lactating mothers, was treated with ivermectin (200 µg/kg) and albendazole (400 mg) once a year in the frame of the Global Programme to Eliminate Lymphatic Filariasis (GPELF), which was implemented from 2001-2006 (Mohammed et al., 2006; 2008). Unpublished data of the MoHSW in Zanzibar confirm that there has been a reduction in prevalence and intensity of soil-transmitted helminthiasis over the years (WHO, 2002a; Mohammed et al., 2008; Stothard et al., 2009).

5.2.6. Drug efficacy in Zanzibar

A study on the efficacy of mebendazole (single oral dose of 500 mg) carried out on Pemba revealed cure rates 21 days post-treatment of 97% for *A. lumbricoides*, 23% for *T. trichiura* and 8% for hookworm. The egg-reduction rate achieved with mebendazole was 99% for *A. lumbricoides*, 81% for *T. trichiura* and 52% for hookworms (Albonico et al., 2003). A study on the efficacy of albendazole (triple oral dose of 400 mg), conducted in schoolchildren from Unguja, revealed cure rates of 99% against *A. lumbricoides*, 43% for *T. trichiura*, 88% for hookworms and 45% for *S. stercoralis*, three weeks post-treatment. The egg-reduction rates were 99% for *A. lumbricoides*, 92% for *T. trichiura* and 99% for hookworm (Marti et al., 1996). In the same study ivermectin (single oral dose of 200 µg/kg) showed cure rates against *A. lumbricoides* of 100%, for *T. trichiura* of 11%, for hookworms of 0% and for *S. stercoralis* of 83%. The egg-reduction rates of ivermectin were 100% for *A. lumbricoides*, 60% for *T. trichiura* and zero for hookworm (Marti et al., 1996). A recent study conducted in Unguja revealed, however, cure rates using albendazole of only 42% and 25% for *A. lumbricoides* and *T. trichiura* infections, respectively (Stothard et al., 2008), indicating a considerable decrease in efficacy of this drug compared to prior studies. A decrease in the efficacy of mebendazole was reported from Zanzibar, too (Albonico et al., 2003). These observations call for a close monitoring of the effectiveness of the drugs used for large-scale application on Zanzibar.

5.2.7. Health education in Zanzibar

Both *Piga vita Kichocho* and the GPELF included health education measures. The “Juma na Kichocho” booklet was used as teaching aid of schistosomiasis in a visually appealing and non-didactic manner, which aimed to educate children by enforcing key health messages set within an appropriate cultural context and story setting (Stothard et al., 2006). The GPELF acted on a much broader level. Informal meetings were held yearly with institutional and political leaders. Posters, information sheets and slogans carrying health messages about lymphatic filariasis were distributed through schools and throughout the island, TV and radio programmes were broadcasted, and local newspapers reported about the GPELF activities and deworming days (WHO, 2002a; Mohammed et al., 2006).

5.2.8. Environmental sanitation in Zanzibar

Sanitation and access to safe water have improved over the last decades in Zanzibar. There are, however large discrepancies between Unguja and Pemba, and between the rural and urban population. According to the household budget survey from 2004/05, 41.4% of rural and 3.8% of urban households in Zanzibar had no access to safe excreta disposal (OCGS, 2006). Stratified by district, in 46% of the households in district North A, 41% in district North B, 19% in district Central, 18% in district South, 8% in district West and 1% in district Urban had no toilet facilities (OCGS, 2006).

Zanzibar entirely depends on underground water as a primary source. Most households in Zanzibar have access to piped water (71%), either at home or from neighbour’s housing units or from community supplies. Another 16% of households use other protected sources than piped water. Unprotected water sources are used by 13% (OCGS, 2006).

The solid waste management in Zanzibar is problematic and critically influencing the health of the population. Only one-quarter of Zanzibar’s solid waste is disposed at a landfill closed to Zanzibar Town. The remaining waste is disposed at un-official sites, incinerated on the ground, consumed by animals, swept away by the rain or blown away by the wind (MoHSW, 2007).

5.3. Diagnosis of soil-transmitted helminth infections

Our knowledge on the epidemiology of various diseases is based on the performance and operational characteristics of the diagnostic methods applied (Bergquist et al., 2009). Routine

diagnosis of soil-transmitted helminths relies on the microscopic identification of eggs or larvae in faecal samples. Adult female worms produce hundreds of eggs per day, which are excreted with the stool. The eggs can be detected and counted with the widely used Kato-Katz thick smear (Katz et al., 1972). The Kato-Katz method is recommended by the WHO as standard measure to evaluate the prevalence and intensity of soil-transmitted helminth infections (Montresor et al., 1998). Advantages of the Kato-Katz method are that it can be performed at all places where a microscope is available, that the kit material is cheap and that most of it can be reused, that the test procedure is easy, and that results are rapidly available (Montresor et al., 1998). Its disadvantage is that only a tiny amount of stool can be examined (41.7 mg), which negatively impacts on the test sensitivity, especially when infection intensities are low (Booth et al., 2003). Additionally, the Kato-Katz method fails to detect *S. stercoralis* larvae (Steinmann et al., 2007). Also the ether-concentration technique on sodium acetate-acetic acid-formalin (SAF) – or formalin (5% or 10%) – preserved stool specimens, routinely used for intestinal protozoa but also applicable for soil-transmitted helminth diagnosis, has a low sensitivity for *S. stercoralis* (Sukhavat et al., 1994; Steinmann et al., 2007). Hence, more complicated techniques such as the Baermann (García and Bruckner, 2001) or the Koga agar plate (Koga et al., 1991) need to be employed for *S. stercoralis* diagnosis. These methods are rarely used, and therefore *S. stercoralis* has recently been termed the most neglected of the neglected tropical diseases (Olsen et al., 2009).

Since the sensitivity of parasitological methods is related to the intensity of infection (Pit et al., 1999), it will become more difficult to accurately assess the epidemiological situation of soil-transmitted helminth infections in areas where control programmes are successfully progressing from morbidity control towards transmission control and finally local elimination. One solution to improve the sensitivity of, for example, the Kato-Katz method is to examine multiple stool samples per patient or to examine multiple sub-samples of the same stool (Nielsen and Mojon, 1987; Booth et al., 2003; Steinmann et al., 2007). Since this solution is not ideal, more sensitive parasitological methods than the Kato-Katz are desired. A promising new technique for the diagnosis of soil-transmitted helminth infections is the FLOTAC method (Cringoli, 2006; 2010; Utzinger et al., 2008). This flotation method allows the quantification of eggs and/or larvae of nematodes in up to 1 g of faeces. Therefore its sensitivity for the detection of helminths is supposed to be higher, especially in situations of low infection intensities (Cringoli, 2006). First investigations showed that the FLOTAC technique detected more hookworm cases than the Kato-Katz thick smear; although light infections were occasionally missed (Utzinger et al., 2008). Further in-depth studies on the

performance of the FLOTAC technique for the diagnosis of soil-transmitted helminths and its suitability for local helminth control programmes and drug efficacy evaluations are urgently required and the thesis presented here made a step in this regard.

Serological tests as the enzyme-linked immuno-sorbent assay (ELISA) can also be used for the diagnosis of soil-transmitted helminth infections, including *S. stercoralis*. They are, however, invasive techniques (require blood), cumbersome to perform and need a certain level of laboratory infrastructure (Snowden and Hommel, 1991; van Doorn et al., 2007). Therefore they have only limited applicability in resource-poor countries where soil-transmitted helminth infections are endemic. Additionally, cross-reacting antibodies between intestinal helminth and filarial worms are a common phenomenon, which impacts negatively on the test specificities (Maizels et al., 1985; Muck et al., 2003; van Doorn et al., 2007). Finally, the detection of helminth DNA in stool samples using real-time PCR provides a specific and sensitive tool for detecting low-grade, patent helminth infections (Markus and Fincham, 2007; Verweij et al., 2007; 2009). However, also this method requires expensive and extensive equipment and will thus not become a routine diagnostic tool in most endemic countries for the near future (Verweij et al., 2009).

Noteworthy, the WHO advocates “preventive” chemotherapy without prior diagnosis to high risk populations in endemic countries since, due to the high costs of microscopes, laboratory material and trained personnel, individual diagnosis is more expensive than treatment (Albonico et al., 2006; WHO, 2006b). However, moving from morbidity control over prevalence control to transmission control with the final goal of elimination of soil-transmitted helminthiases, individual diagnosis will become inevitable. Cost-effective diagnostic methods, which are straightforward to use in helminth control programmes, and detect a wide range of parasitic infections with a constant high sensitivity and specificity are hence to be developed.

5.4. References

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6. Identified research needs

Nowadays, rising efforts for the control of soil-transmitted helminth infections aim at reducing poverty and long-term disability and therefore contribute to the millennium development goals (MDGs) (Hotez et al., 2005; Hotez et al., 2007). However, the large-scale use of anthelmintic drugs, e.g. albendazole, mebendazole and ivermectin, besides its huge benefits in reducing disease burden, also bears a risk for the development of resistance (Albonico et al., 2004; Osei-Atweneboana et al., 2007). To assess the benefits of large-scale deworming and in order to recognize emerging resistance, close monitoring of long-term treatment programmes is of prime importance (Hotez et al., 2007). Hence, the epidemiology of soil-transmitted helminths, including *S. stercoralis*, as well as risk factors for infection and re-infection regularly need to be re-assessed in settings undergoing long-term large-scale anthelmintic chemotherapy, i.e. in Zanzibar. For the sensitive detection of soil-transmitted helminth infections, especially in areas where prevalences and infection intensities are decreasing as a result of ongoing interventions, improved diagnostic approaches are essential. Finally, monitoring of drug efficacy and the evaluation of new compounds or drug combinations are of major importance to sustain successful helminth control.

7. Goals

The three goals of this PhD thesis were (i) to compare and evaluate different diagnostic techniques for the detection of soil-transmitted helminth infections, (ii) to determine the current epidemiology and risk factors of soil-transmitted helminth infections, including *S. stercoralis*, in environmentally and socio-economically distinct settings in Zanzibar, and (iii) to assess the long-term effect of periodic anthelmintic treatment on soil-transmitted helminth prevalences and infection intensities, and on the efficacy of drugs. The following specific objectives were related to these three goals.

7.1. Specific objectives

(i) Comparison and evaluation of different diagnostic techniques:

- To elucidate the effect of repeated stool sampling and the use of the Kato-Katz, Baermann and Koga agar plate method in areas targeted for preventive chemotherapy that are characterized by low-infection intensities.

- To compare single and multiple Kato-Katz thick smears with a single FLOTAC examination for diagnosing *A. lumbricoides*, hookworm and *T. trichiura* infections in schoolchildren from Unguja, Zanzibar, an area that has been targeted for annual anthelmintic treatment since 2003.
- To summarize experiences gained thus far with the FLOTAC technique for diagnosis of human helminth infections.
- To investigate the sensitivity and performance of the FLOTAC method when applied on-site in the Helminth Control Laboratory Unguja (HCLU) in an anthelmintic drug efficacy trial, and to compare the cure rates and egg reduction rates revealed with the Kato-Katz and FLOTAC method.

(ii) Current epidemiology and risk factors of soil-transmitted helminth infections in Zanzibar:

- To assess the prevalence and intensity of soil-transmitted helminth infections, placing particular emphasis on *S. stercoralis*, among children from selected madrassas (Koran schools) and primary schools in the six districts of Unguja and to relate the spatial distribution of soil-transmitted helminth infections to known geological features.
- To determine patterns and risk factors of helminth infections and anaemia in a rural and a peri-urban community on Unguja in the context of national helminth control programmes.

(iii) Long-term effect of periodic anthelmintic treatment:

- To determine the prevalence and intensity of soil-transmitted helminth infections, including *S. stercoralis*, among children from Chaani and Kinyasini primary school on Unguja and to compare the results with data from 1994 obtained in the same schools, in order to study the dynamics of soil-transmitted helminth infections in the face of preventive chemotherapy.
- To assess the current efficacy and safety of albendazole and mebendazole administered alone or in combination with ivermectin against *T. trichiura* and other soil-transmitted helminth infections in two primary schools on Unguja, Zanzibar.

8. Study sites

The Zanzibar archipelago belongs to the United Republic of Tanzania in East Africa and consists of the two main islands, Unguja and Pemba, and various smaller islands. The main economies of Zanzibar are the export of coconuts, cloves, chillies, copra and seaweed. Besides agriculture, fishing is another important economic activity of Zanzibar's population. In the past years, tourism has emerged as a possible successor to the ailing clove industry. There are two annual wet seasons in Zanzibar: the Masika rains from the south lasting usually from mid-March to mid-June, and the Vuli rains from north-east occurring during November and December. The climate is tropical with an average annual temperature of 27°C. On Zanzibar, anthelmintic drugs have been administered to schoolchildren and the whole population on a regular basis for more than a decade. For this reason Zanzibar is a precursor in soil-transmitted helminth control and a most appropriate platform for the evaluation of measures of future use in the control of soil-transmitted helminths.

The field sites of this PhD work were located on Unguja, which is a low-lying island of coral formation. The 1,666 km² of Unguja are administratively divided in six districts with a projected total population of 1,155,065 in 2007 (OCGS, 2007). The 6 districts are structured in small administrative areas (shehias). A shehia consists of 1-3 villages and is headed by a government official or headman called sheha.

Two cross-sectional surveys were carried out in June and July 2007. The first study included children visiting madrassas (Koran schools) in five districts (North A, North B, West, Central, South) and in five primary schools of the Urban district (Figure 6). The second study was carried out in Chaani and Kinyasini primary schools in district North A. In June and July 2008, a cross-sectional survey was performed including inhabitants of all age-groups of the peri-urban shehia Dole (district West) and of the rural shehia Bandamaji (district North A). In March-May 2009, a randomized controlled trial was carried out in the primary schools of Kinyasini and Kilombero (district North A).

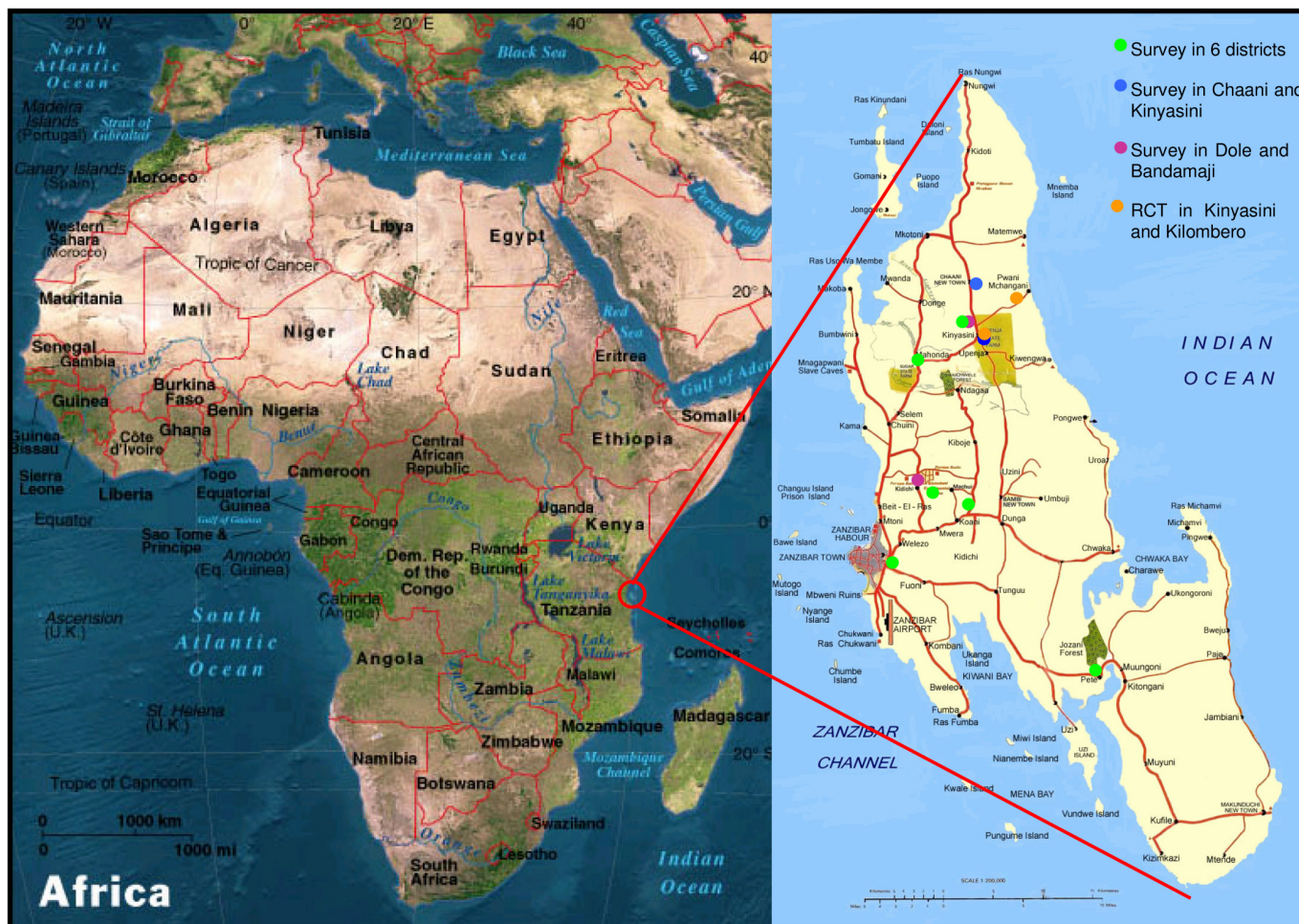


Figure 6. Map of Unguja, Zanzibar, and its location in Tanzania, Africa. (sources: <http://www.nationalgeographic.com/resources/ngo/maps/view/images/africam.jpg>; <http://www.shoortravel.com/zanzibarmap.html>; accessed: 02.03.2010).

RCT: Randomised controlled trial

**9. Diagnosis of soil-transmitted helminths in the era of preventive chemotherapy:
effect of multiple stool sampling and use of different diagnostic techniques**

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9.1. Abstract

Background: Soil-transmitted helminth infections are common throughout the tropics and subtropics and they disproportionately affect the poorest of the poor. In view of a growing global commitment to control soil-transmitted helminthiasis, there is a need to elucidate the effect of repeated stool sampling and the use of different diagnostic methods in areas targeted for preventive chemotherapy that are characterized by low-infection intensities. In this study, we focused on schoolchildren on Unguja Island, Zanzibar; an area where anthelmintic drugs have been repeatedly administered over the past decade.

Methodology/Principal Findings: Three serial stool samples from each of 342 schoolchildren were examined using the Kato-Katz (K-K), Koga agar plate (KAP), and Baermann (BM) techniques. These methods were used individually or in combination for the diagnosis of *Ascaris lumbricoides* (K-K), *Trichuris trichiura* (K-K), hookworm (K-K and KAP), and *Strongyloides stercoralis* (KAP and BM). The examination of multiple stool samples instead of a single one resulted in an increase of the observed prevalence; e.g., an increase of 161% for hookworm using the K-K method. The diagnostic sensitivity of single stool sampling ranged between 20.7% for BM to detect *S. stercoralis* and 84.2% for K-K to diagnose *A. lumbricoides*. Highest sensitivities were observed when different diagnostic approaches were combined. The observed prevalences for *T. trichiura*, hookworm, *A. lumbricoides* and *S. stercoralis* were 47.9%, 22.5%, 16.5% and 10.8% after examining 3 stool samples. These values are close to the ‘true’ prevalences predicted by a mathematical model.

Conclusion/Significance: Rigorous epidemiologic surveillance of soil-transmitted helminth infections in the era of preventive chemotherapy is facilitated by multiple stool sampling bolstered by different diagnostic techniques.

Keywords: Soil-transmitted helminthiasis, *Ascaris lumbricoides*, hookworm, *Strongyloides stercoralis*, *Trichuris trichiura*, diagnosis, mass drug administration, Zanzibar

9.2. Author Summary

Diseases caused by parasitic worms inflict an enormous public health burden in developing countries. There is a growing effort to control worms with drugs. The success of repeated drug administrations can be assessed by measuring the decline in the prevalence and intensity of worm infections. Accurate diagnosis is a challenge, especially in areas with low infection intensities. We studied the effect of stool sampling efforts and the use of different diagnostic techniques on the measured prevalence of worms, including hookworms, large intestinal roundworms (*Ascaris lumbricoides*), whipworms (*Trichuris trichiura*) and dwarf threadworms (*Strongyloides stercoralis*) in Zanzibar, where worm control has been implemented over the past decade. Three early morning stool samples were collected from each of 342 schoolchildren on 3 consecutive days and analyzed with different techniques. The observed prevalence of the different worms increased with an enhanced sampling effort and when different diagnostic methods were combined. Examination of 3 stool samples per individual resulted in prevalences of *T. trichiura*, hookworm, *A. lumbricoides* and *S. stercoralis* of 47.9%, 22.5%, 16.5% and 10.8%, respectively. To conclude, the examination of multiple stool samples and the use of different techniques are recommended for accurate diagnosis of worms in areas undergoing repeated mass drug administration.

9.3. Introduction

Soil-transmitted helminth infections inflict a significant burden on the world's poorest populations living in rural or deprived urban settings in developing countries (Bethony et al., 2006; WHO, 2006a). The most prevalent soil-transmitted helminths are *Ascaris lumbricoides*, *Trichuris trichiura* and the hookworms (*Ancylostoma duodenale* and *Necator americanus*), each parasitizing hundreds of millions of people (Bethony et al., 2006; Hotez et al., 2007; Hotez et al., 2008). Pre-school as well as school-aged children and pregnant women are the groups at highest risk of morbidity due to these infections (Montresor et al., 2002; Goodman et al., 2007). *Strongyloides stercoralis* is another important human helminth species, with disseminated infections being potentially fatal (Carvalho and Da Fonseca Porto, 2004; Vadlamudi et al., 2006).

Significant progress has been made in the control of soil-transmitted helminthiasis by means of large-scale administration of anthelmintic drugs targeting high-risk groups or entire populations. A number of initiatives to reduce helminth-related morbidity are currently underway in different countries (Savioli et al., 2004). Single-dose anthelmintic treatment, usually without prior diagnosis administered to high-risk groups, is the strategy of choice. This approach has been termed 'preventive chemotherapy' (WHO, 2006b). It is important to note, however, that cure is often not complete and depends on the anthelmintic drug utilized (Keiser and Utzinger, 2008). The predominance of light infections following anthelmintic drug administration is deemed acceptable because worm load has been convincingly linked with morbidity (Bethony et al., 2006). Additionally, a decreased number of worms results in a decline of egg excretion and, hence, in reduced environmental contamination and transmission. For both reasons the success of mass drug administration is more accurately measured if infection intensities rather than prevalences are observed (Bundy et al., 1992). The most widely used approach to assess the prevalence and infection intensity of the major soil-transmitted helminths (i.e., *A. lumbricoides*, the hookworms and *T. trichiura*) is the Kato-Katz (K-K) technique (Katz et al., 1972), which is also recommended by the World Health Organization (WHO) (Montresor et al., 1998). However, the K-K method lacks sensitivity if only a single stool sample is examined, particularly in areas with high proportions of light-intensity infections (Booth et al., 2003). A small number of helminth eggs, unequally excreted over days and patchily distributed in stool, can occasionally not be detected in the small amount of stool examined with the K-K (i.e., 41.7 mg), hence negatively impacting on the method's sensitivity. For the detection of *S. stercoralis*, other and more labor-, material- and

infrastructure-demanding methods than the K-K technique are required, turning *S. stercoralis* into a particularly neglected helminth (Steinmann et al., 2007). *S. stercoralis* larvae hatch in the intestines of humans, and infections are most sensitively identified with the Koga agar plate (KAP) method (Koga et al., 1991) and the Baermann (BM) technique (García and Bruckner, 2001). However, the true sensitivity of different diagnostic approaches used to detect *S. stercoralis* infections is still debated (de Kaminsky, 1993; Marchi Blatt and Cantos, 2003; Steinmann et al., 2007).

The aim of this study was to investigate the performance of the K-K, KAP and BM techniques, as well as combinations thereof, for the diagnosis of soil-transmitted helminth infections in an area exposed to intensive helminth control activities. The study focused on schoolchildren in two settings of Zanzibar, an island where helminth control programs, emphasizing chemotherapy-based morbidity control, have been carried out since the mid-1990s (Renganathan et al., 1995; Mohammed et al., 2008; Stothard et al., 2008). In a cross-sectional survey multiple stool samples were collected and examined with the above-mentioned methods to assess the effect of sampling effort and the use of multiple techniques for helminth-specific diagnosis.

9.4. Materials and methods

9.4.1. Study area and population

The study was carried out on Zanzibar Island (Unguja), Tanzania, in June and July 2007. The average annual temperature in Unguja is 26.5°C. There is a long rainy season lasting from mid-March to mid-June, and a wet period with short rains from October to December.

Stool samples were obtained from children attending the primary schools of Kinyasini and Chaani, where a number of previous surveys revealed a high prevalence and infection intensity of soil-transmitted helminths (Marti et al., 1996; Stothard et al., 2000; Rudge et al., 2008). Both schools are located in the district “North A”, ~35 km northeast from Zanzibar Town, and are served by the national helminth control program which has been administering anthelmintic drugs on a fairly regular basis to school-aged children since the mid-1990s using single-dose mebendazole (500 mg) or albendazole (400 mg). For this survey, 30-50 schoolchildren from each of the 7 standards (grades) were randomly selected and invited to participate.

9.4.2. Field and laboratory procedures

Participating schoolchildren were asked to submit 3 stool specimens over consecutive days. Specimens were collected in the early morning. Within 3 hours, the specimens were transported to the Helminth Control Laboratory Unguja (HCLU) located in Mianzini, Zanzibar Town, where diagnosis was initiated immediately. Specimens were processed and examined by experienced laboratory technicians from HCLU. Each specimen was investigated according to the following priorities. First, for the detection of helminth eggs, a single K-K thick smear (Katz et al., 1972) was prepared on microscope slides using the standard template holding 41.7 mg feces. After a clearing time of 40-60 min, the slides were examined under a light microscope. The number of helminth eggs was counted on a per-species basis, and recorded. For quality control, a random sample of 5% of all slides was re-examined by a senior laboratory technician.

Second, for the detection of *S. stercoralis* and hookworm larvae, the KAP procedure was performed (Koga et al., 1991). For this purpose, agar plates were freshly prepared every evening, and stored at 4°C. A groundnut-sized portion of a stool sample (~2 g) was placed in the middle of the agar plate. The closed Petri dish was incubated in a humid chamber for 2 days at ambient temperature. Following incubation, the plates were examined for the presence of *S. stercoralis* and hookworm larvae under a light microscope. The larvae of hookworm are usually more inert and tend to stay close to where the stool sample has been placed on the agar, whereas *S. stercoralis* larvae are more active and mobile. However, discriminative characteristics can only be determined under a microscope. Hence, the plates were rinsed with 10 ml of a 10% acetyl-formalin solution. The eluent was centrifuged at 500 g for 1 min, and the sediment was microscopically examined at 400 x magnification. Hookworm and *S. stercoralis* were determined on the basis of established morphologic characteristics, i.e., the long buccal cavity and small genital primordium of hookworm larvae, and the short buccal cavity and large genital primordium of rhabditiform (L₁) *S. stercoralis* larvae. The filariform larvae (L₃) of the latter nematode can be identified by their characteristically forked tail.

Third, the BM technique (García and Bruckner, 2001) was used for *S. stercoralis* detection. For this purpose, a walnut-sized stool sample (~10 g) was placed on a gauze inserted into a glass funnel, and covered with tap water. The apparatus was then exposed to artificial light directed to the bottom of the funnel. After 2 hours, the bottom 50 ml of the liquid was collected in a plastic tube, and spun at 500 g for 2 min. The supernatant was removed using a water suction pump. The sediment was transferred to a microscope slide, and

examined under a microscope at a 100 x magnification to detect, and a 400 x magnification to confirm the identity of *S. stercoralis* L₁ larvae.

9.4.3. Data management and statistical analysis

All data were entered twice in Microsoft Excel version 10.0 (2002 Microsoft Corporation). Datasets were compared using EpiData version 3.1 (EpiData Association; Odense, Denmark), and discrepancies removed based on the original records.

Statistical analyses were carried out with JMP version 5.0.1 (SAS Institute; Cary, NC, United States of America). Only schoolchildren who had 3 stool samples examined with the same method or combination of methods were included in the final analyses. The number of eggs counted in the K-K thick smear was multiplied by a factor of 24 to obtain a standard infection intensity measure, which is expressed in eggs per gram of stool (EPG). The arithmetic mean EPG for each individual was calculated to summarize the EPG of stool samples submitted by the same individual. The arithmetic means were used to stratify the *A. lumbricoides*, hookworm and *T. trichiura* infection intensities according to guidelines put forward by WHO (Montresor et al., 1998). The thresholds for moderate and heavy infections were 5000 and 50,000 EPG for *A. lumbricoides*, 1000 and 10,000 EPG for *T. trichiura*, and 2000 and 4000 EPG for hookworm, respectively.

The geometric mean EPG for the whole study population was calculated taking into account both positive and negative readings of the K-K using the 10th logarithm of the EPG augmented by 1 ($\log(n+1)$).

The sensitivity (i.e., proportion of true positives identified as positive) and negative predictive value (i.e., proportion of healthy people among negative test results) of the individual diagnostic tools and of appropriate combinations were assessed. Species-specific ‘true’ prevalences and the number of stool samples were also estimated to attain a given percentage of false negatives using the mathematical model developed by Marti and Koella (Marti and Koella, 1993). This model employs the frequency of positive test results among stool samples submitted by the same individual to predict the sensitivity of the diagnostic test and to calculate the number of stool samples needed for the test to be below a given percentage of false negative results. The procedure follows an approach developed by Mullen and Prost (Mullen and Prost, 1983), and has been employed before to predict the ‘true’ prevalences of soil-transmitted helminths, including *S. stercoralis* (Bogoch et al., 2006; Steinmann et al., 2007; 2008).

9.4.4. Ethical considerations and treatment

This study was embedded in a school-based parasitological survey in Unguja, which is regularly conducted by the HCLU. Approval for the study was given by the institutional research commission of the Swiss Tropical Institute (Basel, Switzerland) and the National Health Service Local Research Ethics Committee (application 03.36) of St. Mary's Hospital (London, United Kingdom) on behalf of the Natural History Museum/Imperial College London. The study protocol was cleared by the WHO (Geneva, Switzerland), the Ministry of Health and Social Welfare (Stone Town, Zanzibar) and the Ministry of Education of Unguja (Stone Town, Zanzibar).

The headmasters of the Kinyasini and Chaani primary schools were informed about the purpose and procedures of the study. The schoolchildren were then informed by the teachers. Parents or legal guardians had given written informed consent to all anticipated medical interventions including parasitological surveys at school level when registering their child for school attendance. The children were treated regardless of their infection status with a single-dose albendazole (400 mg) within the framework of the annual mass drug administration conducted by the HCLU. Children with confirmed *S. stercoralis* infections were treated with ivermectin (single-dose, 200 µg/kg).

9.5. Results

9.5.1. Study cohort

Figure 7 shows that among the 401 children selected for the study in Kinyasini and Chaani schools, 221 (55.1%) were girls and 180 (44.9%) were boys. The children were aged between 7 and 20 years. The median age was 12 years and 80% of the children were between 9 and 14 years. Overall, 342 children submitted 3 stool samples over consecutive days, resulting in a compliance rate of 85.3%. Among them, 340 individuals (99.4%) had 3 samples examined with the K-K method, 318 (93.0%) with the KAP method, and 292 (85.4%) with the BM method. Since the combination of the KAP and K-K techniques (for hookworm detection) and the combination of the KAP and the BM methods (for *S. stercoralis* detection) were of particular interest, the analysis focused on 316 (97.5%) and 277 (81.0%) schoolchildren, respectively. Complete data records, i.e., 3 stool samples examined with all 3 diagnostic tests, were available for 277 out of 401 individuals, resulting in an overall compliance rate of 69.1%.

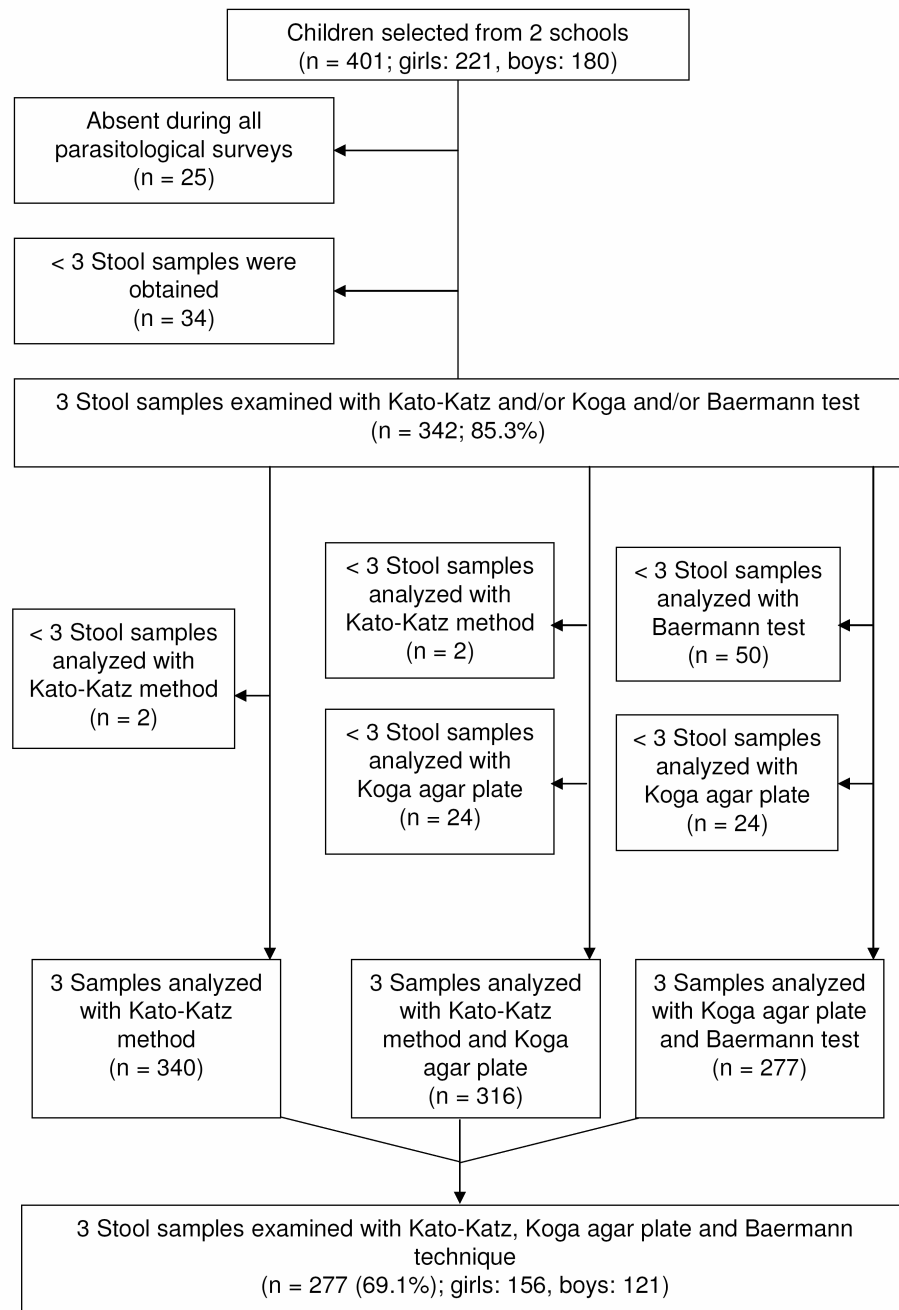


Figure 7. Flow chart detailing the study participation and compliance of randomly selected children from Chaani and Kinyasini schools, Zanzibar. Those children who provided 3 stool samples were included in the final analysis. The final cohort comprised those children who had complete data records, i.e., 3 stool samples examined with 3 different diagnostic methods.

9.5.2. Parasitological findings, stratified by diagnostic method

The observed intestinal helminth prevalences according to different techniques and combinations thereof, and in relation to the number of stool samples examined, are summarized in Figure 8. While the examination of 3 rather than a single stool sample by the K-K method lead to a rather small increase in the number of individuals considered *A. lumbricoides*-positive (from 13.5% to 16.5%; +22%), large increases were observed for *T. trichiura* (from 25.9% to 47.9%; +85%) and, most conspicuously, for hookworm (from 7.1% to 18.5%; +161%).

A similar trend was observed for hookworm and *S. stercoralis* when results from the KAP tests were considered. The examination of 3 instead of a single stool sample by the KAP method resulted in increases of the observed prevalences of both hookworm and *S. stercoralis* of more than 80% (from 7.9% to 14.5% for hookworm, and from 3.5% to 6.3% for *S. stercoralis*). Regarding the BM method, the analysis of 3 stool samples raised the prevalence of *S. stercoralis* from 3.4% (first sample) to 7.2% (all 3 samples; +112%).

The occurrence of hookworm and *S. stercoralis* was investigated by a combination of 2 different methods. The use of the KAP method increased the number of hookworm infections diagnosed by the K-K technique by a factor of 1.2, detecting an additional 11 infections. For *S. stercoralis*, the number of the BM-positives was surpassed by a factor of 1.5 or an additional 10 infections if the KAP results were also considered.

Using the data of 3 stool specimens, analyzed with each method or combination of methods, and Marti and Koella's mathematical model (Marti and Koella, 1993) revealed 'true' prevalences. Prevalences were calculated of 50.1% (standard deviation (SD) = 5.8%) for *T. trichiura*, and 16.5% (SD = 4.0%) for *A. lumbricoides* based on 3 K-K thick smears; 24.2% (SD = 5.2%) for hookworm using 3 K-K plus 3 KAP; and 15.8% (SD = 7.0%) for *S. stercoralis* employing 3 KAP plus 3 BM tests.

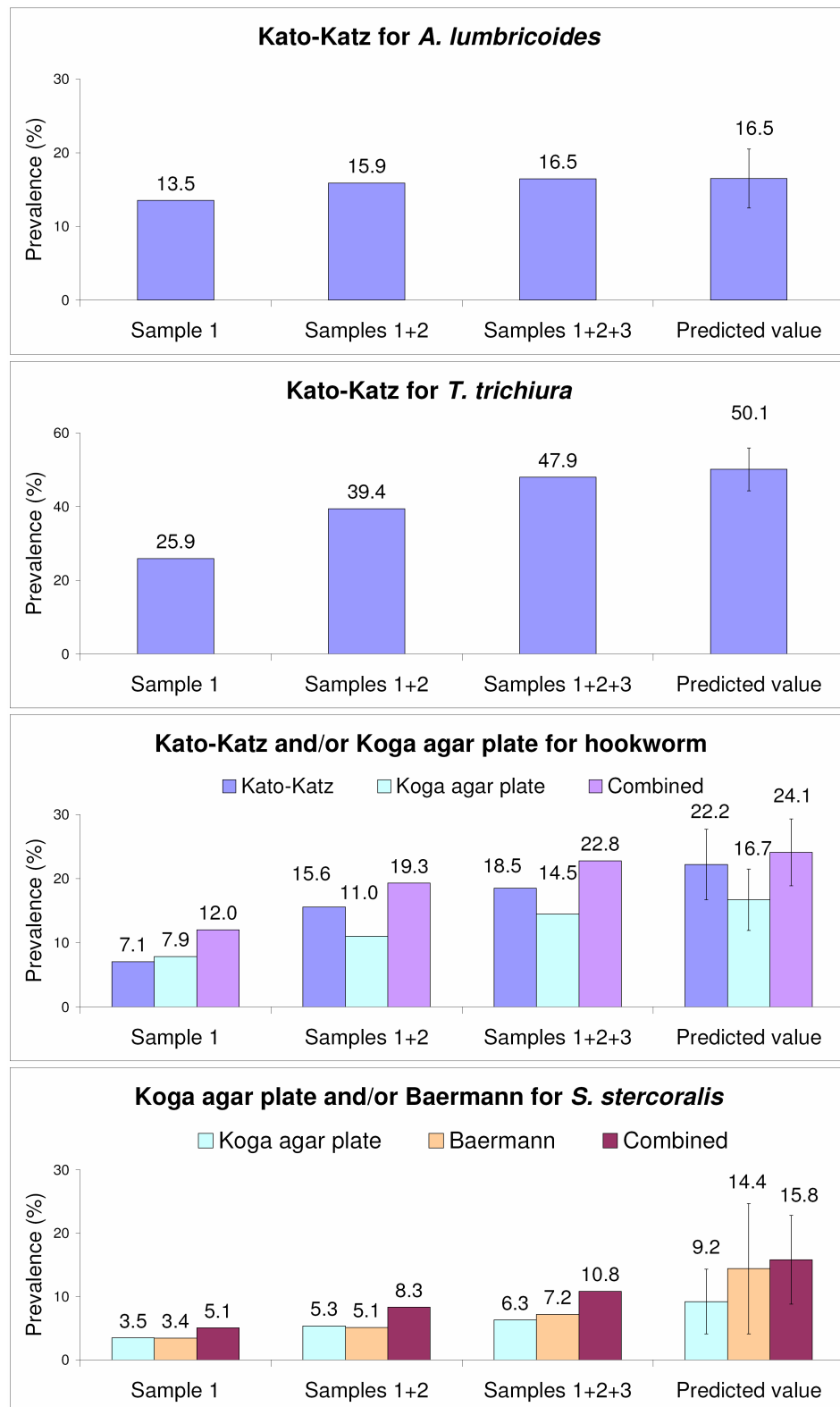


Figure 8. Diagrams detailing the differences in the observed and estimated ‘true’ prevalence of soil-transmitted helminth infections employing different diagnostic methods in relation to the number of stool samples from children from Chaani and Kinyasini schools, Zanzibar.

9.5.3. Infection characteristics

The population geometric mean EPG values for *A. lumbricoides*, hookworm and *T. trichiura* obtained with the K-K method are summarized in Table 2, together with the maximum EPG found in a single stool sample and the infection intensities among the positives, stratified by common intensity classes. According to the WHO-defined infection intensity classification (Montresor et al., 1998), most infected schoolchildren included in the final analyses had low-intensity infections; 98.4% for hookworm (1-1999 EPG), 98.1% for *T. trichiura* (1-999 EPG) and 73.2% for *A. lumbricoides* (1-4999 EPG). No high-intensity infections were found for any of the soil-transmitted helminths. Children with moderate infection intensity of *A. lumbricoides* (5000-49,999 EPG) *T. trichiura* (1000-9999 EPG) and hookworm (2000-3999 EPG) were consistently diagnosed positive in all 3 stool samples, whereas light-intensity infections were sometimes diagnosed in only 1 or 2 of the 3 samples.

The majority of *S. stercoralis* infections (85.0%) were diagnosed in only 1 of the 3 stool samples examined with the BM technique. Three children had 2 positive samples, but only 1 child provided 3 positive samples.

Table 2. Characteristics of soil-transmitted helminth infections among children from Chaani and Kinyasini schools, Zanzibar, as determined by the Kato-Katz technique.

Parasite species	No. of children examined	No. (%) of children infected	Geometric mean (EPG) ^a	Maximum EPG count	No. of infected children stratified by infection intensity (values in brackets are percentage, %)			Method
					Light	Moderate	Heavy	
<i>T. trichiura</i>	340	162 (47.7)	0.77	2880	159 (98.1)	3 (1.9)	0 (0.0)	Kato-Katz
Hookworm	340	63 (18.5)	0.22	2400	62 (98.4)	1 (1.6)	0 (0.0)	Kato-Katz
<i>A. lumbricoides</i>	340	56 (16.5)	0.53	17,520	41 (73.2)	15 (26.8)	0 (0.0)	Kato-Katz

^a EPG = eggs per gram of feces based on Kato-Katz thick smear examination

9.5.4. Performance of the diagnostic methods

As shown in Table 3, the sensitivity of the diagnostic methods rose considerably when 3 stool samples were examined instead of a single one. The highest sensitivities were observed for the diagnosis of *A. lumbricoides* (99.6%) and *T. trichiura* (95.1%) when analyzing 3 stool samples with the K-K method. Also for hookworm and *S. stercoralis* detection, the employed methods showed highest sensitivities when 3 stool samples were examined. The combination of K-K plus KAP for hookworm diagnosis had a markedly higher sensitivity (93.1%) compared to either the K-K (83.3%) or the KAP method alone (86.8%). The combination of KAP plus BM for the identification of *S. stercoralis* showed an increased sensitivity compared to the BM technique (sensitivity of 68.5% versus 50.0%) but was equal to the sensitivity of the KAP method alone (68.5%). The negative predictive value of all employed methods and method combinations was above 92% if 3 stool samples were analyzed.

The samples size needed, if up to 1% false negative results were considered acceptable, was 3 and 5 samples for *A. lumbricoides* and *T. trichiura* using the K-K method, 8 and 7 samples for hookworm with the K-K or KAP method, respectively, and 12 or 20 stool samples for *S. stercoralis* with the KAP or BM method, respectively. Combining the latter 2 methods, 5 and 12 stool samples were necessary for the diagnosis of hookworm and *S. stercoralis*, respectively.

Table 3. Sensitivity of individual and combined diagnostic methods if 1 or 3 stool samples from children from Chaani and Kinyasini schools, Zanzibar, were examined (all values expressed as percentage, %) and samples needed to obtain $\leq 1\%$ false negative test results.

	Kato-Katz method	Koga agar plate method	Baermann method	Kato-Katz plus Koga agar plate method	Koga agar plate plus Baermann method
<i>A. lumbricoides</i>					
Sensitivity of method (3 samples)	99.6	—	—	—	—
Sensitivity of individual test (SD)	84.2 (5.8)	—	—	—	—
Negative predictive value	99.9	—	—	—	—
Samples needed if $\leq 1\%$ false negatives are allowed	3	—	—	—	—
<i>T. trichiura</i>					
Sensitivity of method (3 samples)	95.1	—	—	—	—
Sensitivity of individual test (SD)	63.4 (5.0)	—	—	—	—
Negative predictive value	95.3	—	—	—	—
Samples needed if $\leq 1\%$ false negatives are allowed	5	—	—	—	—
Hookworm					
Sensitivity of method (3 samples)	83.3	86.8	—	93.1	—
Sensitivity of individual test (SD)	45.0 (9.3)	49.0 (10.6)	—	59.0 (7.9)	—
Negative predictive value	95.5	97.4	—	97.9	—
Samples needed if $\leq 1\%$ false negatives are allowed	8	7	—	5	—
<i>S. stercoralis</i>					
Sensitivity of method (3 samples)	—	68.5	50.0	—	68.5
Sensitivity of individual test (SD)	—	32.0 (16.8)	20.7 (15.4)	—	32.0 (13.7)
Negative predictive value	—	96.9	92.3	—	94.4
Samples needed if $\leq 1\%$ false negatives are allowed	—	12	20	—	12

9.6. Discussion

There is a paucity of high-quality data regarding the effect of stool sampling effort and the use of different techniques for the diagnosis of soil-transmitted helminths in different epidemiologic settings. In particular, information is lacking on the performance of widely employed diagnostic tools in the current era of preventive chemotherapy (WHO, 2006b). Our findings were obtained from investigating fecal samples from more than 300 schoolchildren in Zanzibar. Children were screened over multiple days using 3 different fecal examination methods and our results confirm that an increased sampling effort and the use of multiple diagnostic approaches result in higher observed helminth prevalences (Booth et al., 2003; Steinmann et al., 2007; 2008). For example, while the observed hookworm prevalence was only 7.1% after examination of a single K-K thick smear, the cumulative prevalence after screening 3 stool samples with the K-K method was more than twice as high (18.5%). Using both the K-K and the KAP method on 3 stool samples resulted in an observed prevalence of 22.8%. This observed prevalence is close to the modeled prevalence of 24.1%. The effect of multiple sampling on the observed prevalence is also notable for *S. stercoralis* and *T. trichiura*. It was, however, much less obvious with regard to the detection of *A. lumbricoides*.

Since the measured prevalence of individual helminth species increased considerably as a function of sampling effort, the diagnostic sensitivity of single stool samples may be insufficient. Indeed, the examination of only 1 stool sample considerably underestimated *T. trichiura*, hookworm and *S. stercoralis* infections. If 3 stool samples were examined, the sensitivity of all tests increased and the negative predictive values were consistently above 90%. The low sensitivity of single tests in the current setting can be attributed to the predominantly light infections among the pupils of Chaani and Kinyasini schools. Both schools are covered by the national helminth control program, which has distributed single-dose mebendazole (500 mg) and albendazole (400 mg) once or several times yearly since 1995 and 2003, respectively. Additionally, the repeated rounds of mass administration of ivermectin and albendazole as part of the global program to eliminate lymphatic filariasis, which has been implemented since 2001 (Mohammed et al., 2006; 2008) and targets the whole island of Unguja, has also impacted on the worm load in children. The administration of ivermectin (200 µg/kg) through the latter program has most probably had a considerable impact on *A. lumbricoides* and *S. stercoralis*, since ivermectin exhibits significant ascaricidal

and strongyloidicidal activity. Another factor lowering the sensitivity of diagnostic tests is day-to-day variation in fecal egg output (Hall, 1981; Anderson and Schad, 1985).

It is also known that the detection of hookworm eggs is influenced by delays in stool processing (Dacombe et al., 2007) and, when using the K-K technique, the time from slide preparation to reading under a microscope (WHO, 1994). In this study, slides were examined within 40-60 min after preparation, which is the upper limit of the recommended clearing time (WHO, 1994), especially if the warm climate is taken into account. The time from stool production in the early morning until the fecal samples reached the bench – at least 3 hours – might also have impacted on the diagnostic sensitivity. Moreover, there was considerable variation between individuals and from one day to another. A time delay of more than 3 hours from stool production to examination reduced the sensitivity of the K-K method for hookworm diagnosis by almost 50% in a recent study carried out in Malawi (Dacombe et al., 2007). The KAP method also allows hatching of hookworm larvae. Thus, the KAP technique can supplement the K-K method and, in the present study, the combination of these 2 methods yielded a sensitivity of 93.1% after examination of 3 stool samples per individual. A higher sensitivity for the KAP method was found compared to the K-K technique for the diagnosis of hookworm, which is in agreement with recent observations made in China (Steinmann et al., 2007). However, the KAP procedure requires some basic laboratory infrastructures that are often not available in developing country settings, multiple days for incubation in a humid chamber and trained laboratory personnel (Koga et al., 1991).

It is conceivable that in some cases *S. stercoralis* larvae failed to leave the stool sample placed in the middle of the agar plate (KAP) or on the gauze embedded in the glass funnel (BM). This will result in false-negative diagnoses as the larvae can only be detected when moving on the surface of the agar plate or settling at the bottom of the funnel. Hence, relying on stool examination only will result in a certain number of false negatives. Moreover, *S. stercoralis* larvae can replicate within the host and autoinfection is possible without larvae being excreted, thus not all infections can be detected by parasitological techniques. It has been suggested that multiple stool sampling and the combination of several diagnostic methods reveal *S. stercoralis* infections with the highest sensitivity (Dreyer et al., 1996). The observed increase in sensitivity obtained by combining the BM and the KAP methods coincides with results of de Kaminsky (1993), but is in contrast with a recent study done by Steinmann and colleagues (2007) where the BM technique identified ‘all’ cases. However, we recommend the concurrent use of both methods as each of them has strengths and limitations. Regarding the KAP method, it is not easy to perform under field conditions and requires

expertise in differentiating *S. stercoralis* from hookworm larvae and, potentially, also from environmental nematodes. Additionally, agar plates containing infective larvae pose a biohazard and need to be handled and disposed of with care. Regarding the BM technique, it is less time consuming and detected larvae can be identified more easily. The most notable disadvantage of this method is the large quantity of stool needed, and hence compliance is an issue.

The calculated number of stool samples needed to reach a rate of $\leq 1\%$ false negative diagnoses resulted in high numbers except for *A. lumbricoides*, where 3 samples subjected to the K-K method were sufficient. For accurate diagnosis of *T. trichiura*, hookworm and *S. stercoralis*, it was found that up to 20 stool samples need to be examined in this low intensity setting. This is not feasible except for specialized small-scale studies. Therefore, diagnostic methods with higher sensitivity and low technical demands are urgently needed. Sero-diagnosis of soil-transmitted helminths might be an option, but this approach has some disadvantages such as its more invasive nature (i.e., blood collection), the persistence of antibodies after treatment and potential cross-reactivity with other nematodes. The non-invasive FLOTAC technique (Cringoli, 2006; Utzinger et al., 2008) holds promise to fill this gap and a broad-scale validation of this tool for species-specific helminth diagnosis is underway.

We conclude that in epidemiologic settings characterized by low-infection intensities of soil-transmitted helminths, it is important to examine multiple stool samples in order to avoid underestimating the 'true' prevalence of soil-transmitted helminth infections, and hence their transmission potential. Our results indicate that for rigorous epidemiologic surveillance, a combination of methods is required to more accurately assess the situation. From a more general infectious diseases perspective, our observations could potentially better pinpoint interactions between helminthiasis and other tropical diseases (e.g., malaria), which are likely modulated by chronic worm infections even of low egg/larvae output (Markus and Fincham, 2007). The discovery, development and deployment of new tools for the diagnosis and quantification of soil-transmitted helminth infections, including *S. stercoralis*, is of considerable importance for successful helminth control, and remains a research priority (Hotez et al., 2006; Gasser et al., 2008; Ramanathan et al., 2008).

9.7. Acknowledgments

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9.9. Translation of abstract into German

Diagnose von durch Bodenkontakt übertragenen Wurmerkrankungen in der Ära präventiver Chemotherapie: Bedeutung von wiederholten Stuhluntersuchungen und unterschiedlichen diagnostischen Methoden

Hintergrund: Infektionen mit durch Bodenkontakt übertragenen Würmern treten in den Tropen und Subtropen häufig auf und belasten besonders die ärmsten Bevölkerungsgruppen. In Hinsicht auf die wachsenden weltweiten Bemühungen diese Wurmerkrankungen einzudämmen muss der Nutzen von wiederholten Stuhluntersuchungen und die Anwendung von unterschiedlichen diagnostischen Methoden in Gebieten, in welchen präventive Chemotherapie angewandt wird und welche somit von niedriger Infektionsintensität gekennzeichnet sind, ermittelt werden. In dieser Studie legen wir den Schwerpunkt auf die Untersuchung von Schulkindern aus Unguja, Sansibar, einer Insel, auf welcher Antiwurm-Medikamente im letzten Jahrzehnt regelmäßig verabreicht wurden.

Methoden/wichtigste Ergebnisse: Drei Stuhlproben in Folge wurden von 342 Schulkindern eingesammelt und mit der Kato-Katz (K-K), Koga Agarplatten (KAP) und Baermann (BM) Methode untersucht. Diese Methoden wurden einzeln oder in Kombination für die Diagnose von *Ascaris lumbricoides* (K-K), *Trichuris trichiura* (K-K), Hakenwürmern (K-K und KAP) und *Strongyloides stercoralis* (KAP und BM) angewandt. Die Untersuchung von mehreren anstelle einer einzelnen Stuhlprobe führte zu einem Anstieg der registrierten Prävalenz; z.B. einem Anstieg von 161% für Hakenwurminfektionen, wenn die K-K Methode benutzt wurde. Die Sensitivität der Untersuchung einer einzelnen Stuhlprobe bewegte sich zwischen 20.7% für die BM Methode um *S. stercoralis* nachzuweisen, und 84.2% für die K-K Methode um *A. lumbricoides* zu diagnostizieren. Höhere Sensitivitäten konnten beobachtet werden wann immer unterschiedliche diagnostische Methoden kombiniert wurden. Die Prävalenzen für *T. trichiura*, Hakenwürmer, *A. lumbricoides* und *S. stercoralis* betrugen 47.9%, 22.5%, 16.5% und 10.8% wenn 3 Stuhlproben untersucht wurden. Diese Werte liegen nahe der 'wahren' Prävalenzen, die durch ein mathematisches Model vorhergesagt werden.

Schlussfolgerung/Bedeutung: Die genaue epidemiologische Überwachung von Wurminfektionen im Zeitalter der präventiven Chemotherapie wird durch die Untersuchung von mehreren Stuhlproben mit unterschiedlichen Methoden gefördert.

10. A single FLOTAC is more sensitive than triplicate Kato-Katz for the diagnosis of low-intensity soil-transmitted helminth infections

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10.1. Summary

Accurate diagnostic tools are pivotal for patient management and surveillance of helminth control programmes, particularly in the current era of preventive chemotherapy. Three consecutive stool samples were obtained from 279 schoolchildren from Zanzibar, an island where anthelmintic drugs have been administered at large-scale for more than a decade. All stool samples were examined with the Kato-Katz method. Additionally, one sample per child was preserved in sodium acetate-acetic acid-formalin solution, and examined with the FLOTAC technique. Considering the pooled results of both methods as diagnostic ‘gold’ standard, the observed prevalences of *Trichuris trichiura*, hookworm and *Ascaris lumbricoides* were 63.4%, 35.8% and 22.9%, respectively. The sensitivity of a single stool sample examined by FLOTAC for diagnosing *T. trichiura*, hookworm and *A. lumbricoides* was 88.7%, 83.0% and 82.8%, respectively. Lower sensitivities were observed for Kato-Katz even after examining three stool samples; 71.8%, 46.0%, and 70.3% respectively. Kato-Katz revealed considerably higher helminth infection intensities than FLOTAC. The κ agreement between a single FLOTAC and triplicate Kato-Katz was 0.63 for diagnosing *A. lumbricoides*, 0.50 for *T. trichiura*, but only 0.30 for hookworm. The high sensitivity of FLOTAC holds promise for patient management, monitoring soil-transmitted helminth transmission and endpoint(s) of control at the population level.

Keywords: *Ascaris lumbricoides*; Hookworm; *Trichuris trichiura*; FLOTAC; Kato-Katz; Zanzibar

10.2. Introduction

The most common soil-transmitted helminths are *Ascaris lumbricoides*, the hookworms (*Ancylostoma duodenale* and *Necator americanus*) and *Trichuris trichiura*. Globally, more than one billion people are infected with one or several of these intestinal nematodes (Bethony et al., 2006; Albonico et al., 2008; Hotez et al., 2008). Infections can cause chronic and debilitating disease with a global burden that might be as high as 39 million disability-adjusted life years (Hotez et al., 2006).

The regular administration of anthelmintic drugs without prior diagnosis to high-risk groups has become the strategy of choice for the control of helminth infections and the term “preventive chemotherapy” is now widely used (Savioli et al., 2004; Brooker et al., 2006; WHO, 2006). There is growing emphasis on co-administering anthelmintic drugs to populations living in areas where multiple helminths co-exist (Hotez et al., 2006; Lammie et al., 2006; Hotez et al., 2008; Mohammed et al., 2008). Such large-scale interventions are usually school-based as school-aged children generally are at highest risk of soil-transmitted helminth infection and constitute a readily accessible group within the community. Moreover, the beneficial impact of treatment is believed to be greatest during childhood development (Bethony et al., 2006; Brooker et al., 2006).

The evidence-base that repeated mass drug administration significantly reduces the prevalence and morbidity associated with soil-transmitted helminths is compelling (Dickson et al., 2000; Savioli et al., 2004; Kabatereine et al., 2007; Taylor-Robinson et al., 2007). On the Zanzibar islands, periodic school-based drug distribution has been implemented since 1994 using single-dose oral mebendazole (500 mg) and, from 2003 onwards, single-dose oral albendazole (400 mg) (Renganathan et al., 1995; Stothard et al., 2008). Within the frame of the Global Programme to Eliminate Lymphatic Filariasis (GPELF), single-dose oral albendazole (400 mg) plus single-dose oral ivermectin (200 µg/kg) are administered once every year to the whole eligible population in Zanzibar since 2001. These chemotherapy-based control efforts significantly reduced the prevalence and intensity of soil-transmitted helminth infections, and led to a decline in the burden caused by lymphatic filariasis (Mohammed et al., 2008).

As low-intensity helminth infections are often missed if a single stool sample is examined by the widely applied Kato-Katz method (Booth et al., 2003), there is a pressing need for the development and validation of alternative diagnostic tools. These tools should be accurate and user-friendly so that they can be utilized for surveillance of helminth control programmes.

Recently, it has been shown that the FLOTAC technique – a non-invasive multivalent faecal egg-count technique (Cringoli, 2006) – holds promise for the accurate diagnosis of hookworm infections (Utzinger et al., 2008). Since FLOTAC uses a much larger amount of stool for the detection of helminth eggs than the Kato-Katz method (up to 1 g *versus* 41.7 mg) there is a greater chance of detecting helminth eggs, especially if contained in the sample at low density.

The aim of our study was to compare single and multiple Kato-Katz thick smears with a single FLOTAC examination for diagnosing *A. lumbricoides*, hookworm and *T. trichiura* in schoolchildren on the island of Unguja, Zanzibar, an area that has been targeted for annual anthelmintic treatment since 2003.

10.3. Materials and methods

10.3.1. Study area and population

The study was carried out in the primary schools of Chaani and Kinyasini, located in the North of Unguja, the main island of Zanzibar, United Republic of Tanzania, in June and July 2007. A total of 401 schoolchildren were randomly selected and invited to participate in the study.

10.3.2. Ethical considerations and anthelmintic treatment

This study was embedded in the 2007 parasitological school survey carried out annually by the Helminth Control Laboratory of Unguja (HCLU; Stone Town, Zanzibar). The study protocol was cleared by the World Health Organization (WHO; Geneva, Switzerland; OD/TS-07-00331) and the Ministry of Health and Social Welfare (MoHSW) of Unguja (Stone Town, Zanzibar). Written informed consent to parasitological surveys and all related medical interventions at school level in Unguja is routinely obtained from the parents and/or legal guardians at the time children are registered for schooling. School directors were informed about the specific aims of the present study. After obtaining their consent, the purpose and procedure of the study were explained to the participating children. At study completion, all children were given a single 400 mg oral dose of albendazole regardless of their infection status in the frame of the annual mass drug administration conducted by the HCLU.

10.3.3. Field and laboratory procedures

The schoolchildren were invited to submit three early morning stool samples, collected over consecutive days. Filled containers were transferred to HCLU. Processing of stool samples was as follows. First, a single 41.7 mg Kato-Katz thick smear was prepared (Katz et al., 1972). Kato-Katz thick smears were allowed to clear for 40-60 min prior to examination under a microscope by experienced laboratory technicians. The number of helminth eggs was counted and recorded for each helminth species separately. A random sample of 5% of the slides was re-examined by a senior technician for quality control. Second, ~1-2 g of stool from one out of the three consecutively submitted stool samples were transferred to 20 ml plastic tubes, and conserved in 10 ml of a sodium acetate-acetic acid-formalin (SAF) solution (Marti and Escher, 1990) for subsequent FLOTAC examination.

The SAF-preserved stool samples were forwarded to the Department of Pathology and Animal Health, University of Naples “Federico II” (Naples, Italy). After ~6 months, the SAF-preserved samples were processed with the FLOTAC technique (Cringoli, 2006). In brief, each sample was passed through a screen with an aperture of 350 µm in order to remove large fibres, and an additional 10 ml of SAF was added. Equal amounts of this stool-SAF suspension were pipetted into two pre-weighed 15 ml Falcon tubes and centrifuged for 3 min at 170 g using a Hettich centrifuge (Tuttlingen, Germany). The supernatant was discarded and the pellets weighed to the nearest mg using a Gibertini balance (Milan, Italy). Each tube was then filled to the 6 ml mark with one of two flotation solutions, namely (i) flotation solution no. 4 (sodium nitrate: NaNO_3 315 g plus 685 ml H_2O ; specific density = 1.20; designated ‘S4’), and (ii) flotation solution no. 13 (zinc sulphate and mercury II iodine and potassium iodide: $\text{ZnSO}_4 \times 7 \text{H}_2\text{O}$ 600 g plus 600 ml H_2O and KI 78 g plus HgI_2 100 g plus 63 ml H_2O ; specific density = 1.45; designated ‘S13’) (Cringoli et al., 2004). The pellets in the respective solutions were suspended, and 5 ml of the suspension transferred into one of the two chambers of the FLOTAC apparatus, each holding a volume of 5 ml. The apparatus was then centrifuged for 5 min at 120 g. Finally, after the translation of the top portion of the flotation chambers with the FLOTAC apparatus, microscopy of both observation grids at a 100x magnification commenced. Helminth eggs were counted and separately recorded for each species according to the flotation solution used.

10.3.4. Statistical analysis

Data were entered twice in a Microsoft® Excel 2002 spreadsheet (Microsoft Corporation, Redmond, WA, USA). The consistency of the two files was validated using EpiInfo™ version 6.04d (Centres for Disease Control and Prevention; Atlanta, GA, USA).

Statistical analyses were carried out using JMP version 5.0.1 (SAS Institute; Cary, NC, USA) and EpiInfo. Only children with complete data records (i.e. three Kato-Katz thick smears and one FLOTAC) were included into the final analysis. We considered the pooled results from the FLOTAC (single stool sample) and the Kato-Katz thick smears (three consecutive stool samples) as diagnostic ‘gold’ standard. The prevalence of helminth infections, the sensitivity and the negative predictive value (NPV), including 95% confidence intervals (CIs), were calculated for a single FLOTAC examination, and the respective or all three Kato-Katz thick smears. The agreement between the FLOTAC and the triplicate Kato-Katz thick smear readings for the diagnosis of *A. lumbricoides*, hookworm and *T. trichiura* was assessed using κ statistics.(Landis and Koch, 1977)

The number of helminth eggs per gram of faeces (EPG) was obtained by multiplying the number of helminth eggs recorded in the Kato-Katz thick smear by factor 24. The EPG for the FLOTAC technique was estimated for each chamber separately, as follows: $EPG = (\text{helminth-specific egg count} \times 1.2) / (\text{weight of stool pellet and tube} - \text{weight of tube})$. The classification into low, medium and high infection intensity was based on the arithmetic mean EPG derived from the three Kato-Katz readings considering thresholds set forth by the World Health Organization (WHO).(Montresor et al., 1998)

The arithmetic mean EPG and standard error (SE) for the whole study cohort as well as the 25%, 50%, 75% and 90% percentiles of the EPG were calculated considering the results of the triplicate Kato-Katz and the single FLOTAC (both flotation solutions separately). Differences between the three mean EPG values (i.e. FLOTAC according to the two different flotation solutions and the triplicate Kato-Katz results) were analyzed with the Kruskal-Wallis test. Finally, pair-wise comparisons were made and analysed using the Wilcoxon signed rank sum test. Statistical significance was considered at a significance level of 0.05.

10.4. Results

10.4.1. Operational results

Figure 9 shows that of 401 randomly selected schoolchildren in Chaani and Kinyasini, 279 had complete data records, i.e. three stool samples examined with the Kato-Katz method and one SAF-preserved sample additionally examined with the FLOTAC technique, using two different flotation solutions. Hence, the overall compliance was 69.6%. The final study cohort comprised 164 (58.8%) girls and 115 boys (41.2%). The median age was 12 years (range: 7 to 20 years). Reasons for non-compliance were absence during collection days ($n = 25$), submission of only one or two stool samples ($n = 36$), and insufficient quantity of stool for SAF-conservation ($n = 61$).

The mean weight of the SAF-preserved stool-pellets examined with S4 and S13 was 0.84 g (range: 0.19 to 2.61 g) and 0.85 g (range: 0.21 to 2.60 g), respectively.

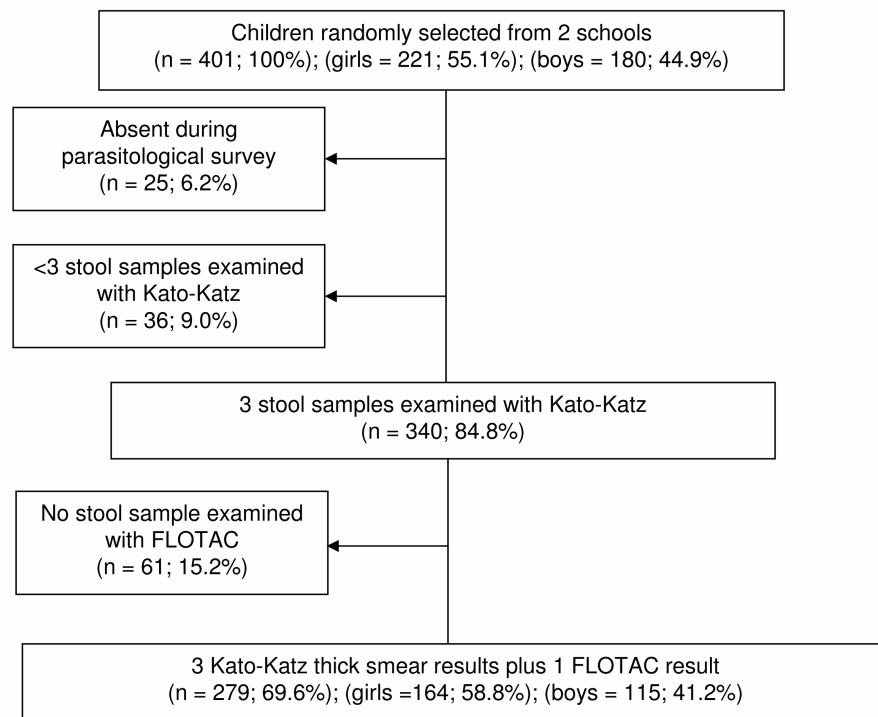


Figure 9. Diagram detailing study participation and stool sample submission compliance of randomly selected children attending Chaani and Kinyasini schools, Zanzibar in mid-2007. All children having 3 stool samples examined with Kato-Katz and out of these 1 stool sample examined with the FLOTAC method were included in the final analyses.

10.4.2. Comparison of methods

Considering the pooled results from three Kato-Katz thick smears and a single FLOTAC as diagnostic ‘gold’ standard, the prevalence was 63.4%, 35.8% and 22.9% for *T. trichiura*, hookworm and *A. lumbricoides*, respectively. The observed prevalence of soil-transmitted helminths increased as a function of higher sampling effort (Figure 10). The examination of three instead of only one Kato-Katz thick smear increased the observed prevalence of hookworm from 5.0% to 16.5% (an increase of 230%), that of *T. trichiura* from 25.8% to 45.5% (+76%) and that of *A. lumbricoides* from 12.9% to 16.1% (+25%). Prevalence estimates based on a single FLOTAC examination (combined results of both flotation solutions) were 56.3% for *T. trichiura*, 29.8% for hookworm and 19.0% for *A. lumbricoides*. These estimates are 81% (for hookworm), 24% (for *T. trichiura*) and 18% (for *A. lumbricoides*) higher than triplicate Kato-Katz results. Differences were observed in diagnosing *T. trichiura* and hookworm according to the flotation solution used in the FLOTAC apparatus. There were slightly more *T. trichiura* infections discovered by S13 (+13%), whereas S4 revealed 5.4-fold more hookworm infections than S13. No difference was observed for *A. lumbricoides* diagnosis with regard to the flotation solution used.

According to the Kato-Katz results and infection intensity thresholds set forth by WHO, all children infected with hookworm, 99.2% of the children infected with *T. trichiura* and 95.6% of those with an *A. lumbricoides* infection were of light intensity. The remainder were categorised as moderate infections. No child was found to be heavily infected with any of the intestinal nematodes.

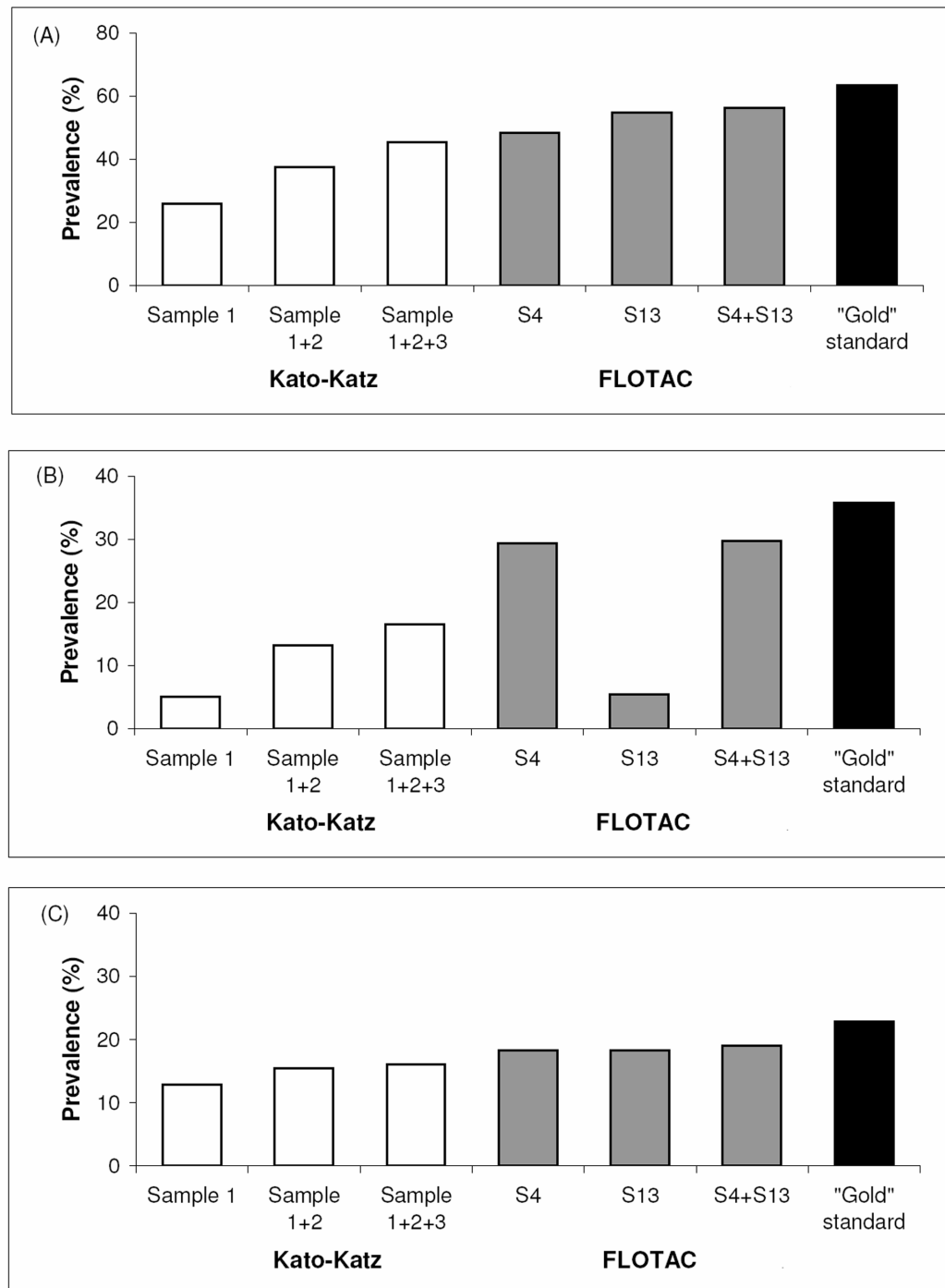


Figure 10. Prevalence of soil-transmitted helminth infections in children from Chaani and Kinyasini schools, Zanzibar, in mid-2007. Results are shown for the first, the first plus the second, and all three Kato-Katz thick smears separately, and for S4, S13 and the combination of both flotation solutions of a single FLOTAC examination. The pooled results of triplicate Kato-Katz plus one FLOTAC test were considered as diagnostic 'gold' standard (n = 279). (A) Kato-Katz and FLOTAC for *Trichuris trichiura*; (B) Kato-Katz and FLOTAC for hookworm; (C) Kato-Katz and FLOTAC for *Ascaris lumbricoides*.

According to our diagnostic ‘gold’ standard, the sensitivity of FLOTAC based on a single stool sample was higher than three Kato-Katz thick smears prepared from three consecutive stool samples for the detection of common soil-transmitted helminths (Table 4). The sensitivity of a single FLOTAC for diagnosing *T. trichiura*, hookworm and *A. lumbricoides* was 88.7%, 83.0% and 82.8%, respectively. Triplicate Kato-Katz resulted in respective sensitivities of 71.8%, 46.0% and 70.3%. Considering a single Kato-Katz, the sensitivity of hookworm diagnosis was only 14.0%. The highest NPV was observed for diagnosing *A. lumbricoides* using the FLOTAC method (95.1%) while the lowest NPV was found for diagnosing *T. trichiura* with a single Kato-Katz thick smear (49.3%).

Table 5 shows the results in two-way contingency table format, comparing FLOTAC from a single stool sample with Kato-Katz thick smears from three consecutive stool samples. Whilst both, a single FLOTAC examination and triplicate Kato-Katz thick smears, identified 107 cases of *T. trichiura*, an additional 50 cases of *T. trichiura* were detected by FLOTAC only and 20 cases of *T. trichiura* were only found after examining three Kato-Katz thick smears. The κ agreement between the two methods for the diagnosis of *T. trichiura* was moderate ($\kappa = 0.50$). Higher additional numbers of hookworm and *A. lumbricoides* were discovered by a single FLOTAC rather than triplicate Kato-Katz (54 *versus* 17 for hookworm, 19 *versus* 11 for *A. lumbricoides*). The κ agreement between the two methods for diagnosing *A. lumbricoides* was substantial ($\kappa = 0.63$) but only fair for hookworm diagnosis ($\kappa = 0.30$). The κ agreement between the two methods for each helminth showed high statistical significance ($P < 0.001$).

Table 4. Sensitivity and negative predictive value (NPV) of the first and all three Kato-Katz thick smears, and single FLOTAC examinations for the diagnosis of soil-transmitted helminths among 279 schoolchildren from Zanzibar. The pooled results of triplicate Kato-Katz plus one FLOTAC test were considered as diagnostic ‘gold’ standard.

	First Kato-Katz thick smear		All three Kato-Katz thick smears		Single FLOTAC (both flotation solutions)	
	Sensitivity (95% CI)	NPV (95% CI)	Sensitivity (95% CI)	NPV (95% CI)	Sensitivity (95% CI)	NPV (95% CI)
<i>A. lumbricoides</i>	56.3% (43.3-68.4%)	88.5% (83.6-92.1%)	70.3% (57.4-80.8%)	91.9% (87.4-94.9%)	82.8% (70.9-90.7%)	95.1% (91.2-97.4%)
<i>T. trichiura</i>	40.7% (33.4-48.3%)	49.3% (42.3-56.3%)	71.8% (64.4-78.1%)	67.1% (59.0-74.4%)	88.7% (82.9-92.8%)	83.6% (75.6-89.5%)
Hookworms	14.0 (8.1-22.7%)	67.5% (61.5-73.1%)	46.0% (36.1-56.2%)	76.8% (70.8-82.0%)	83.0% (73.9-89.5%)	91.3% (86.3-94.7%)

CI: confidence interval

Table 5. Two-way contingency tables showing the agreement between triplicate Kato-Katz thick smears and a single FLOTAC examination (both flotation solutions) for the diagnosis of soil-transmitted helminths in stool samples from 279 schoolchildren from Zanzibar.

		Triplicate Kato-Katz		Total
		Positive	Negative	
<i>A. lumbricoides</i> *	Single FLOTAC			
	Positive	34	19	53
	Negative	11	215	226
	Total	45	234	279
<i>T. trichiura</i> **	Positive	107	50	157
	Negative	20	102	122
	Total	127	152	279
Hookworm***	Positive	29	54	83
	Negative	17	179	196
	Total	46	233	279

*: κ agreement = 0.63 ($P < 0.001$), indicating a substantial agreement.

** : κ agreement = 0.50 ($P < 0.001$), indicating a moderate agreement.

***: κ agreement = 0.30 ($P < 0.001$), indicating a fair agreement.

Table 6 shows that the Kato-Katz method yielded considerably higher mean EPG estimates than FLOTAC (regardless of the flotation solution) for each helminth species investigated. Indeed, the mean EPGs derived from FLOTAC and triplicate Kato-Katz showed a highly statistically significant difference for *T. trichiura* ($P < 0.001$) and hookworm ($P < 0.001$). A significantly higher mean EPG count for hookworm was obtained with S4 compared to S13 ($P < 0.001$). No significant differences in the mean EPG for *A. lumbricoides* were found between FLOTAC and Kato-Katz and between the two flotation solutions used by FLOTAC, respectively.

In addition to the three common soil-transmitted helminths, eggs of *Enterobius vermicularis* and *Hymenolepis nana* were also diagnosed both by FLOTAC and the Kato-Katz technique. The κ agreement between a single FLOTAC and triple Kato-Katz for the diagnosis of *E. vermicularis* was fair ($n = 5$; $\kappa = 0.30$; $P < 0.001$), derived from one case detected with both methods and two cases each detected by either method. There was a single case of *H. nana*, and it was identified by both methods. In four stool samples the FLOTAC revealed larvae that were most likely *Strongyloides stercoralis*. No *S. stercoralis* larvae were detected by the Kato-Katz method.

Table 6. EPG values (expressed as arithmetic mean [AM], standard error [SE], percentiles and maximum) and their statistically significant differences as revealed by a single FLOTAC (two different flotation solutions used, designated S4 and S13) and triplicate Kato-Katz thick smears for the diagnosis of soil-transmitted helminths in stool samples from schoolchildren from Zanzibar (n = 279).

	AM	SE	Percentile				Maximum	Kruskal-Wallis-test
			25%	50%	75%	90%		P-value
<i>A. lumbricoides</i>								
FLOTAC-S4	40.9	55.7	0.0	0.0	0.0	108.1	2526.3	0.302
FLOTAC-S13	63.4	55.7	0.0	0.0	0.0	275.9	2087.0	
3 Kato-Katz	278.2	32.2	0.0	0.0	0.0	600.0	14760.0	
<i>T. trichiura</i>								
FLOTAC-S4	26.2	9.8	0.0	0.0	5.3	28.9	2016.0	0.001*
FLOTAC-S13	22.1	9.8	0.0	1.4	8.1	33.3	2143.3	
3 Kato-Katz	51.0	5.7	0.0	0.0	24.0	124.8	2880.0	
Hookworm								
FLOTAC-S4	3.4	2.7	0.0	0.0	1.76	5.3	273.5	0.001*
FLOTAC-S13	0.4	2.7	0.0	0.0	0.0	0.0	88.7	
3 Kato-Katz	12.2	1.6	0.0	0.0	0.0	0.0	720.0	

*: statistically significant difference ($P < 0.05$)

#: Standard error uses a pooled estimate of error variance

10.5. Discussion

With the further scaling up of preventive chemotherapy targeting common helminthic diseases, the prevalence and intensity of infection and morbidity are expected to decline in many places. As the widely used Kato-Katz method lacks sensitivity to detect low-intensity soil-transmitted helminth infections (Booth et al., 2003; Knopp et al., 2008), there is a pressing need for more accurate, yet simple, user-friendly and non-invasive diagnostic tools (Peeling et al., 2006). Here, we have shown that in a random sample of schoolchildren from Zanzibar, the examination of a single stool sample with the FLOTAC method more accurately diagnosed infections with the three main soil-transmitted helminths than the examinations of three consecutive stool samples with the Kato-Katz technique. Our study further underscores the low sensitivity of a single Kato-Katz thick smear in detecting low-intensity hookworm infections and, to a lesser extent, also that of *T. trichiura* and *A. lumbricoides*. However, neither of the employed diagnostic tools was able to detect *all* helminth infections.

Combining the results of a single FLOTAC and triplicate Kato-Katz thick smears, the observed prevalence of *T. trichiura*, hookworm and *A. lumbricoides* were 63.4%, 35.8% and 22.9%, respectively. The sensitivity of a single FLOTAC ranged between 82.8% (for *A. lumbricoides* diagnosis) and 88.7% (for *T. trichiura* diagnosis), and was considerably higher than the sensitivity of triplicate Kato-Katz examinations (range: from 46.0% for hookworm diagnosis to 71.8% for *T. trichiura* diagnosis).

The remarkably low sensitivity of a single – and even triplicate – Kato-Katz for the detection of hookworm infections is alarming. The use of glycerol in the Kato-Katz method that could destroy hookworm eggs over time might, at least partially, explain this observation. However, bench aids edited by WHO suggest reading of Kato-Katz thick smears within 30 to 60 min after preparation to remedy this issue (WHO, 1994), and our examinations were within this time frame. Interestingly, the hookworm prevalence obtained in this setting of Unguja was even higher than our expectations based on previous findings (Stothard et al., 2008). The sensitivity of a single FLOTAC for hookworm diagnosis was considerably higher than the sensitivities of single and triple Kato-Katz smears. Compared to a recent investigation employing the FLOTAC technique for the first time in human parasitology that revealed a sensitivity of 88.2%, a slightly lower sensitivity was noted in the present investigation (83.0%) (Utzinger et al., 2008). As the overall hookworm infection intensity according to multiple Kato-Katz thick smears was 155.8 EPG in Côte d'Ivoire, but only 12.2 EPG in Zanzibar, it is conceivable that the somewhat lower sensitivity of FLOTAC for hookworm

diagnosis in the present study is mainly due to the lower mean infection intensity. Indeed, the sensitivity of the Kato-Katz and other coprological tools is also influenced by the intensity of infection (Hall, 1982; Lin et al., 2008).

Although the FLOTAC had a superior diagnostic sensitivity compared to multiple Kato-Katz thick smears, it failed to detect some cases. Since these were diagnosed in the same or consecutive stool samples by the Kato-Katz method only, the κ coefficient indicated only substantial to fair agreement between techniques. In view of this observation, the Kato-Katz method might be considered as an important complement to the FLOTAC method, for example in drug efficacy trials or sentinel surveillance of helminth control programmes.

In our study, the advantage of examining several-fold larger amounts of stool with the FLOTAC apparatus than the widely used Kato-Katz method was most evident for the diagnosis of light-intensity infections with *T. trichiura* and hookworm. Because these helminths produce fewer eggs than *A. lumbricoides*, the probability of detecting their eggs in a larger amount of stool is higher than in a smaller one. However, the reasons why a single FLOTAC resulted in several-fold lower hookworm and *T. trichiura* EPGs compared to Kato-Katz must be investigated. This issue had been stressed already in our preceding work, focussing on hookworm diagnosis among schoolchildren in Côte d'Ivoire.¹⁷ It is also worth mentioning that the two flotation solutions used in the current study performed differently for the identification of hookworm infections. Specifically, S13 revealed a lower hookworm mean EPG value than flotation solution S4. Indeed, S13 underperformed in the diagnosis of hookworm, yielding only 15 cases compared to 82 infections detected with S4.

The identification of a single *H. nana* infection both by the FLOTAC apparatus and the Kato-Katz method indicates the potential of FLOTAC also for the detection of tapeworm infections. Interestingly, *E. vermicularis* was identified by the FLOTAC apparatus in three children but only a poor κ agreement was found with the Kato-Katz test results. However, care is indicated when using the κ statistics for comparison of diagnostic methods for rare parasitic infections. Since *E. vermicularis* infections can only occasionally be detected in stool samples, the method of choice to diagnose *E. vermicularis* remains the Scotch cellophane tape method (Beaver, 1949). The helminth larvae found in four stool samples are most likely *S. stercoralis* larvae, as this helminth is endemic on Unguja island (Marti et al., 1996; Knopp et al., 2008). However, the present configuration of the FLOTAC apparatus does not allow the differentiation of species-specific characteristics (e.g. large genital primordium and short buccal cavity of *S. stercoralis* larvae). A modified FLOTAC apparatus allowing parasite diagnosis at 400x magnification is currently under development.

Our results show that the FLOTAC method holds promise for more accurate diagnosis than the Kato-Katz technique, especially in epidemiological settings characterized by low-intensity helminth infections. Hence, FLOTAC might emerge as a suitable method for the surveillance of helminth control programmes, monitoring of soil-transmitted helminth transmission and verification of local elimination. The higher sensitivity might reveal that the ‘true’ soil-transmitted helminth prevalences in certain low infection intensity settings are considerably higher than currently estimated. This could have a direct impact on policies and control programmes based on proposed WHO treatment algorithms. Higher prevalence estimates derived with the FLOTAC method would, for example, lead to a higher fraction of communities arbitrarily defined as ‘category II’ (i.e. prevalence of infection above 50% and low infection intensities), for which mass drug administration is recommended (Montresor et al., 1998). Higher prevalences of low-intensity helminth infections could also have far-reaching consequences on burden estimates because recent studies point to significant morbidity associated with even light infection intensities (Ezeamama et al., 2005).

The encouraging results obtained with the FLOTAC method for diagnosis of common soil-transmitted helminths call for further development and sequential validation steps. An issue that warrants particular investigation is to elucidate reasons for the significant differences observed in mean hookworm and *T. trichiura* infection intensities when comparing FLOTAC with Kato-Katz. Once the technique is fully validated in different epidemiological settings the hope is to transfer to more basic field laboratories.

In conclusion, our results underscore previous observations which unanimously point to a low sensitivity of traditional and widely used parasitological methods to detect low-intensity soil-transmitted helminth infections. A single FLOTAC identified a higher percentage of ‘all’ cases than three Kato-Katz thick smears from consecutive stool samples. Thus, after further steps of standardization and validation, the FLOTAC could become a supportive diagnostic tool for patient management, and particularly for the rigorous surveillance of helminth control programmes.

10.6. Acknowledgments

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11. FLOTAC: a promising technique for detecting helminth eggs in human faeces[✱]

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11.1. Summary

There is a tendency of neglecting diagnostic issues in the era of ‘preventive chemotherapy’ in human helminthiasis. However, accurate diagnosis cannot be overemphasized for adequate patient management and monitoring of community-based control programmes. Implicit is a diagnostic dilemma: the more effective interventions reduce helminth egg excretion, the less sensitive direct parasitological tests become. Here, experiences gained thus far with the FLOTAC technique for diagnosing common soil-transmitted helminth infections are summarized. A single FLOTAC has higher sensitivity than multiple Kato-Katz thick smears in detecting low-intensity infections. Further validation of the FLOTAC technique in different epidemiological settings is warranted, including diagnosis of intestinal schistosomiasis and food-borne trematodiasis.

Keywords: FLOTAC; Kato-Katz; *Ascaris lumbricoides*; hookworm; *Trichuris trichiura*; diagnosis; Côte d’Ivoire; Zanzibar.

11.2. Introduction

The key strategy for reducing the morbidity caused by soil-transmitted helminth infections (*Ascaris lumbricoides*, hookworm and *Trichuris trichiura*) in endemic countries is large-scale administration of anthelmintic drugs to high-risk groups, often without prior diagnosis. This strategy is termed ‘preventive chemotherapy’ (WHO, 2006). As a result of successful helminth control programmes, infection rates and intensities diminish, and hence direct diagnostic techniques become less sensitive (Bergquist et al., 2009). Here, we summarize our experiences gained thus far with a new faecal egg-count method – the FLOTAC technique (Cringoli, 2006) – for diagnosis of human helminth infections. Comparison is made between a single FLOTAC and multiple Kato-Katz thick smears (Katz et al., 1972).

11.3. Methods

So far, we have been involved in three studies using FLOTAC for human helminth diagnosis; one in Zanzibar (Knopp et al., 2009) and two in Côte d’Ivoire (Utzinger et al., 2008; Glinz et al., 2010). The studies followed cross-sectional epidemiological designs and a total of 493 schoolchildren were enrolled. In both Côte d’Ivoire studies a single faecal sample was collected per child, and 2-3 Kato-Katz thick smears using 41.7 mg templates were prepared. In Zanzibar, three consecutive faecal samples were collected and one Kato-Katz thick smear examined per sample. Additionally, ~1 g of stool from each child was preserved in sodium acetate-acetic acid-formalin (SAF) and subjected to the FLOTAC technique. Preserved stool samples from the first Côte d’Ivoire study and from Zanzibar were transferred to Naples, Italy, and processed ~6 months after stool collection. Samples from the second Côte d’Ivoire study were examined on the spot, 10, 30 and 83 days after collection.

Detailed procedures of FLOTAC have been described elsewhere (Cringoli, 2006; Utzinger et al., 2008; Knopp et al., 2009). In brief, in the first Côte d’Ivoire and the Zanzibar study, flotation solutions no. 4 (FS4; sodium nitrate, specific gravity = 1.20) and 13 (FS13; zinc sulphate plus potassium iodomercurate, specific gravity = 1.45) were used. In the second Côte d’Ivoire study an additional washing step with ether was performed to facilitate a clearer reading under the microscope. Here, FS4 and FS7 (zinc sulphate, specific gravity = 1.35) were employed. Eggs per gram of stool (EPGs) for each helminth species were calculated. Combined results (single FLOTAC plus multiple Kato-Katz) were considered as diagnostic ‘gold’ standard.

11.4. Results

Key results from the three studies are summarized in Table 7. The first Côte d'Ivoire study showed that a single FLOTAC is more sensitive than duplicate Kato-Katz for hookworm diagnosis (sensitivity: 88.2% versus 68.4%). These findings were confirmed in Zanzibar: the sensitivity of a single FLOTAC for hookworm diagnosis was 83.0%, whereas triplicate Kato-Katz only had a sensitivity of 46.0%. Importantly, FLOTAC was also more sensitive for the diagnosis of *T. trichiura* and *A. lumbricoides*. In the second Côte d'Ivoire study, FLOTAC had a higher sensitivity than triplicate Kato-Katz for *T. trichiura* diagnosis at each time point and for hookworm diagnosis at days 10 and 30 post-stool conservation, but not at day 83. In this study, FLOTAC was consistently less sensitive than triplicate Kato-Katz for diagnosis of *A. lumbricoides*. While an apparent decline in sensitivity of FLOTAC was observed for hookworm and *A. lumbricoides* diagnosis as a function of stool preservation duration, the sensitivity for *T. trichiura* diagnosis increased from 93.9% to 100%.

Table 7. Arithmetic mean eggs per gram of faeces (EPG), prevalence and sensitivity (including 95% confidence intervals (CI)) of a single FLOTAC and multiple Kato-Katz thick smears as determined in cross-sectional studies with schoolchildren from Côte d'Ivoire and Zanzibar. Combined results from FLOTAC and multiple Kato-Katz thick smears were considered as diagnostic 'gold' standard thereby setting the sensitivity to 100%.

Setting, year	Population sample (age)	Parasite	Diagnostic 'gold' standard	FLOTAC					Kato-Katz			Reference
			Method and sampling effort	Prevalence (%)	Duration of stool preservation in SAF*	Arithmetic mean EPG	Prevalence (%)	Sensitivity (%) [95% CI]	Arithmetic mean EPG	Prevalence (%)	Sensitivity (%) [95% CI]	
Côte d'Ivoire, 2006	102 schoolchildren (6-14 years)	Hookworm	1 FLOTAC plus 2 Kato-Katz (1 faecal sample)	74.5	6 months	37.7	65.7	88.2 [78.2-94.1]	155.8	51.0	68.4 [56.6-78.3]	Utzinger et al. (2008)
Zanzibar, 2007	279 schoolchildren (7-20 years)	<i>T. trichiura</i>	1 FLOTAC plus 3 Kato-Katz (3 faecal samples)	63.4	6 months	24.2	56.3	88.7 [82.9-92.8]	51.0	45.5	71.8 [64.4-78.1]	Knopp et al. (2009)
		Hookworm	1 FLOTAC plus 3 Kato-Katz (3 faecal samples)	35.8	6 months	1.9	29.8	83.0 [73.9- 89.5]	12.2	16.5	46.0 [36.1-56.2]	Knopp et al. (2009)
		<i>A. lumbricoides</i>	1 FLOTAC plus 3 Kato-Katz (3 faecal samples)	22.9	6 months	52.2	19.0	82.8 [70.9-90.7]	278.2	16.1	70.3 [57.4-80.8]	Knopp et al. (2009)
Côte d'Ivoire, 2008	112 schoolchildren (6-15 years)	Hookworm	1 FLOTAC (day 10) plus 3 Kato-Katz (1 faecal sample)	38.4	10 days	8.5	31.3	81.4 [71.2-88.6]	41.4	21.4	55.8 [46.6-65.0]	D. Glinz et al. (2010)
		Hookworm	1 FLOTAC (day 30) plus 3 Kato-Katz (1 faecal sample)	36.6	30 days	2.0	25.9	70.7 [62.3-79.2]	41.4	21.4	58.5 [49.4-67.7]	D. Glinz et al. (2010)
		Hookworm	1 FLOTAC (day 83) plus 3 Kato-Katz (1 faecal sample)	28.6	83 days	1.8	17.0	59.4 [50.3-68.5]	41.4	21.4	75.0 [67.0-83.0]	D. Glinz et al. (2010)
		<i>T. trichiura</i>	1 FLOTAC (day 10) plus 3 Kato-Katz (1 faecal sample)	29.5	10 days	5.0	27.7	93.9 [89.5-98.4]	6.2	12.5	42.2 [33.3-51.6]	D. Glinz et al. (2010)
		<i>T. trichiura</i>	1 FLOTAC (day 30) plus 3 Kato-Katz (1 faecal sample)	32.1	30 days	11.4	32.1	100 [100.0-100.0]	6.2	12.5	38.9 [29.9-47.9]	D. Glinz et al. (2010)
		<i>T. trichiura</i>	1 FLOTAC (day 83) plus 3 Kato-Katz (1 faecal sample)	30.4	83 days	14.6	30.4	100 [100.0-100.0]	6.2	12.5	41.2 [32.1-50.3]	D. Glinz et al. (2010)
		<i>A. lumbricoides</i>	1 FLOTAC (day 10) plus 3 Kato-Katz (1 faecal sample)	21.4	10 days	65.9	13.4	62.5 [53.5-71.5]	1318	19.6	91.7 [86.6-96.8]	D. Glinz et al. (2010)
		<i>A. lumbricoides</i>	1 FLOTAC (day 30) plus 3 Kato-Katz (1 faecal sample)	20.5	30 days	99.7	11.6	56.5 [47.3 -65.7]	1318	19.6	95.7 [91.2-99.4]	D. Glinz et al. (2010)
		<i>A. lumbricoides</i>	1 FLOTAC (day 83) plus 3 Kato-Katz (1 faecal sample)	19.6	83 days	219.7	10.7	54.6 [45.3-63.8]	1318	19.6	100 [100.0-100.0]	D. Glinz et al. (2010)

*SAF: Sodium acetate-acetic acid-formalin

Table 8. Advantages and disadvantages of the Kato-Katz and FLOTAC techniques for diagnosis of soil-transmitted helminth infections.

Kato-Katz technique	FLOTAC technique
<i>Advantages</i>	<i>Advantages</i>
<ul style="list-style-type: none"> • Relatively straightforward to perform • Inexpensive, simple and reusable equipment • Widely used method, hence laboratory technicians are well acquainted with this technique 	<ul style="list-style-type: none"> • Highly sensitive for low-intensity infections • Use of either fresh or preserved faecal samples • High compliance because only a single faecal sample is required • Straightforward microscopic reading of apparatus (clear, bright, lined 18 x 18 mm grid)
<i>Disadvantages</i>	<i>Disadvantages</i>
<ul style="list-style-type: none"> • Lack of sensitivity for low-intensity helminth infections • Hookworm eggs over-clear fast • Use of only fresh faecal samples • Reduced compliance and increased costs if multiple faecal samples are collected • Difficult and time consuming microscopic reading of slides (dirty, dark, no lines, round smears) 	<ul style="list-style-type: none"> • Time-consuming preparation • Expensive equipment • Need of specialized laboratories (e.g. centrifuge) • Additional washing step with ether might negatively impact on helminth egg detection

11.5. Discussion

Our experiences made thus far are that a single FLOTAC is more sensitive than multiple Kato-Katz thick smears for diagnosing low-intensity soil-transmitted helminth infections. Idiosyncrasies of the epidemiological settings might explain some of the discrepancies in diagnostic sensitivity. Moreover, the additional washing step with ether in the second Côte d'Ivoire study warrants further consideration. As expected, Kato-Katz performs well when EPGs are reasonably high. For example, the sensitivity of Kato-Katz in the second Côte d'Ivoire study was higher than the one of FLOTAC for *A. lumbricoides* diagnosis, but lower for *T. trichiura*. In this setting, *A. lumbricoides* infections were comparatively high (mean intensity: 1318 EPG according to triplicate Kato-Katz), but *T. trichiura* infections were of low intensity (6.2 EPG using triplicate Kato-Katz). In Zanzibar, where EPGs of all soil-transmitted helminth infections were low, FLOTAC had a consistently higher sensitivity than multiple Kato-Katz.

The apparent decrease in sensitivity of FLOTAC observed over time in the second Côte d'Ivoire study, particularly for hookworm diagnosis, seems – at first sight – surprising. Indeed, the first Côte d'Ivoire and the Zanzibar study with faecal samples preserved in SAF for ~6 months revealed high sensitivities for hookworm diagnosis. However, in a separate calibration of FLOTAC with samples also stored in SAF for ~6 months and using different

flotation solutions and washing steps with and without ether, it could be shown that hookworm eggs were well detected but only in the absence of ether. Hence, the apparent decline in the sensitivity for hookworm diagnosis with FLOTAC seems not to be a methodological issue of FLOTAC. Instead, we speculate that hookworm eggs are destroyed by SAF preservation and the concurrent use of ether. An important lesson learnt is that helminth-specific eggs must be considered independently when using the FLOTAC technique with respect to (i) the floatation solution, (ii) the use of ether and (iii) the media used for faecal preservation (e.g., SAF or formaldehyde). What is known for one parasite cannot be readily transferred to other parasites.

Advantages and disadvantages of the FLOTAC and Kato-Katz techniques are summarized in Supplementary Table 8. While FLOTAC is highly sensitive for low-intensity soil-transmitted helminth infections, it is considerably more time consuming regarding preparation (Levecke et al., 2009). In terms of equipment, FLOTAC is more expensive than Kato-Katz. However, because of the low sensitivity of a single Kato-Katz, examinations of more than just one thick smear, ideally derived from multiple stool samples, are recommended for obtaining reliable results (Knopp et al., 2008). Obviously, repeated stool sampling reduces patient compliance, and increases overall costs. Economic evaluations of FLOTAC and its diagnostic potential for detection of eggs of other helminths (e.g. *Enterobius vermicularis*, *Schistosoma mansoni* and food-borne trematodes) are underway. Finally, the diagnostic accuracy of FLOTAC in anthelmintic drug efficacy trials is the subject of our current investigations in Zanzibar. In view of the results highlighted here, we feel that FLOTAC might become a viable tool for accurate patient management and rigorous monitoring of drug intervention studies and community-based helminth control programmes.

11.6. Acknowledgements

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12. Diagnostic Accuracy of Kato-Katz and FLOTAC for Assessing Anthelmintic Drug Efficacy

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12.1. Abstract

Background: Sensitive diagnostic tools are required for an accurate assessment of prevalence and intensity of helminth infections in areas undergoing regular deworming, and for monitoring anthelmintic drug efficacy. We compared the diagnostic accuracy of the Kato-Katz and FLOTAC technique in the frame of a drug efficacy trial.

Methodology/Principal Findings: Stool samples from 343 Zanzibari children were subjected to duplicate Kato-Katz thick smears and the FLOTAC basic technique in a baseline screening in early 2009. The FLOTAC showed a higher sensitivity than the Kato-Katz method for the diagnosis of *Trichuris trichiura* (95% vs. 88%, $p = 0.012$) and *Ascaris lumbricoides* (88% vs. 68%, $p = 0.098$), but a lower sensitivity for hookworm diagnosis (54% vs. 81%, $p = 0.006$). Considering the combined results from both methods as ‘gold’ standard, the prevalences of *T. trichiura*, hookworm and *A. lumbricoides* were 71% (95% confidence interval (CI): 66-75%), 22% (95% CI: 17-26%) and 12% (95% CI: 8-15%), respectively. At follow-up, 3-5 weeks after 174 among the 269 re-examined children were administered anthelmintic drugs, we observed cure rates (CRs) against *A. lumbricoides*, hookworm and *T. trichiura* of 91% (95% CI: 80-100%), 61% (95% CI: 48-75%) and 41% (95% CI: 34-49%), respectively, when using the Kato-Katz method. FLOTAC revealed lower CRs against *A. lumbricoides* (83%, 95% CI: 67-98%) and *T. trichiura* (36%, 95% CI: 29-43%), but a higher CR against hookworm (69%, 95% CI: 57-82%). These differences, however, lacked statistical significance. Considerable differences were observed in the geometric mean fecal egg counts between the two methods with lower egg reduction rates (ERRs) determined by FLOTAC.

Conclusion/Significance: Our results suggest that the FLOTAC technique, following further optimization, might become a viable alternative to the Kato-Katz method for anthelmintic drug efficacy studies and for monitoring and evaluation of deworming programs. The lower CRs and ERRs determined by FLOTAC warrant consideration and could strategically impact on future helminth control programs.

Keywords: FLOTAC; Kato-Katz; *Ascaris lumbricoides*; hookworm; *Trichuris trichiura*; diagnosis; Zanzibar.

12.2. Author Summary

In areas where parasitic worm infections have been successfully reduced as a result of deworming campaigns, the level of infections and drug efficacy must be carefully monitored. For this purpose, diagnostic methods with a high sensitivity are needed. We compared the accuracy of the widely used Kato-Katz method with the more recently developed FLOTAC technique for the diagnosis of parasitic worms. Our study was done with children on Zanzibar island, Tanzania, within the frame of an anthelmintic drug efficacy study. We collected stool samples from 343 children in two primary schools before and after treatment and examined the stool samples with both methods. FLOTAC showed a higher sensitivity than Kato-Katz for the diagnosis of roundworm and whipworm, but a lower sensitivity for hookworm diagnosis. The cure rates determined by FLOTAC were lower for roundworm and whipworm when compared with Kato-Katz. The opposite was found for hookworm. Egg reduction rates were generally lower when the FLOTAC technique was used. Our results suggest that the FLOTAC method, after additional optimization, can become a viable alternative to the Kato-Katz method for anthelmintic drug efficacy studies and for monitoring and evaluation of deworming programs.

12.3. Introduction

Current estimates suggest that soil-transmitted helminths might still affect over a quarter of the world's population and inflict a huge public health burden, particularly on rural and deprived urban communities in the developing world (Hotez et al., 2008; Keiser and Utzinger, 2010; King, 2010). Efforts are underway to reduce morbidity due to soil-transmitted helminthiasis, with an ambitious target to administer anthelmintic drugs regularly to at least 75% and up to 100% of school-aged children and other high-risk groups (WHO, 2002). One region where this goal was already met in 2006 is Zanzibar, a group of islands forming part of the United Republic of Tanzania (WHO, 2008). Indeed, helminth control programs have been implemented in Zanzibar for 20 years and have resulted in marked decreases in prevalence, intensity and morbidity due to soil-transmitted helminthiasis, urinary schistosomiasis, and lymphatic filariasis (Savioli et al., 1989; Mohammed et al., 2006; Knopp et al., 2009b; Stothard et al., 2009).

The diagnosis of soil-transmitted helminth infections with direct parasitological methods based on egg detection in stool is unreliable among infected individuals who harbor only a few intestinal worms, since egg output is much lower than among heavily infected individuals. Hence, in settings where helminth control programs have been implemented and infection intensities dropped as a result of regular deworming, diagnostic methods with a high sensitivity are needed for an accurate assessment of the actual prevalence and intensity of soil-transmitted helminth infections (Bergquist et al., 2009). Sensitive diagnostic tools are also mandatory for monitoring drug efficacy and to detect the emergence and spread of drug resistance (Albonico et al., 2004; Hotez et al., 2007). The Kato-Katz method (Katz et al., 1972) is a widely used diagnostic tool, which provides estimates of population prevalence and infection intensity, and facilitates anthelmintic drug efficacy assessment in clinical trials and monitoring and evaluation of community-based control programs. However, due to the small amount of stool examined (i.e., 41.7 mg), the Kato-Katz method shows a low sensitivity when infection intensities are light, which is common after deworming (Booth et al., 2003). The sensitivity can be improved by examining multiple Kato-Katz thick smears produced from a single stool sample or by examining multiple stool samples (Ebrahim et al., 1997; Knopp et al., 2008; Steinmann et al., 2008).

With the recently developed FLOTAC method, up to 1 g of stool can be examined – 24 times more than with a single Kato-Katz thick smear – and standard protocols are now available (Cringoli et al., 2010). The FLOTAC technique showed a higher sensitivity than

multiple Kato-Katz thick smears for the diagnosis of soil-transmitted helminth infections in previous studies (Utzinger et al., 2008; Knopp et al., 2009a; Knopp et al., 2009c; Glinz et al., 2010). Moreover, FLOTAC outperformed the McMaster technique qualitatively and quantitatively for hookworm diagnosis (Rinaldi et al., 2009). What is not known, though, is how the FLOTAC technique performs within anthelmintic drug efficacy trials, and whether it might be utilized for monitoring and evaluation of helminth control programs that are shifting the tactic from morbidity control to infection and transmission control, and eventually local elimination.

The objective of this study was to compare the accuracy of the Kato-Katz method with the FLOTAC basic technique for the detection of soil-transmitted helminth infections within a randomized controlled trial on anthelmintic drug efficacy and safety carried out in Zanzibar in early 2009 (Knopp et al., 2010). The diagnostic accuracy of each method was determined before and after treatment and anthelmintic drug efficacy was estimated according to the method used.

12.4. Materials and methods

12.4.1. Ethics statement

The study presented here was embedded in a randomized controlled trial with the protocol being reviewed by the institutional research commission of the Swiss Tropical and Public Health Institute (Basel, Switzerland). Ethical approval was given by the ethics committee of Basel (EKBB, reference no. 13/09) and the Ministry of Health and Social Welfare of Zanzibar (MoHSW, reference no. ZAMEC/0001/09). The study is registered at controlled-trials.com, identifier ISRCTN08336605.

The directors and teachers of the primary schools in Kinyasini and Kilombero were informed about the purpose and procedures of the study. The study was explained in lay terms to the school children in their local language (Kiswahili). An informed consent sheet, including study information and the fact that participation was voluntary, was distributed to each child. Written informed consent was obtained from parents or guardians prior to stool sampling. Additionally, oral assent was sought from children.

At the end of the study in May 2009, all children attending the two schools were offered free anthelmintic drugs, as part of the regular deworming done by the Helminth Control Laboratory Unguja (HCLU). Single oral doses of albendazole (400 mg) and praziquantel (40

mg/kg) were administered to school-aged children for preventing morbidity due to soil-transmitted helminthiasis and urinary schistosomiasis, respectively.

12.4.2. Study area and population

The study was carried out on Unguja island, Zanzibar, in the first half of 2009 within the frame of a randomized controlled trial to assess the efficacy and safety of different anthelmintic drugs against *Trichuris trichiura* and other soil-transmitted helminth infections (Knopp et al., 2010). For the assessment of anthelmintic drug efficacy according to the diagnostic method used, we aimed at enrolling at least 200 *T. trichiura*-positive individuals (Albonico, 2003). In view of the sample size calculations for our clinical trial, we aimed at screening 2000 children to identify 600 *T. trichiura*-infected subjects, assuming a prevalence of 30% (Knopp et al., 2009b). All children attending the primary schools of Kilombero and Kinyasini in district North A, located 30-40 km from Zanzibar Town, were eligible to submit stool samples. To reach the number of 200 *T. trichiura*-infected children for assessing anthelmintic drug efficacy, not only with the Kato-Katz, but also with the FLOTAC technique, we systematically preserved ~1 g of every third stool sample collected at the baseline screening for subsequent FLOTAC examinations. Since the *T. trichiura* prevalence turned out to be >50%, we stopped recruiting children during our baseline survey after we had included 1240 children in the study. Among these children, 1066 (86%) had submitted one stool sample; 750 from Kinyasini and 316 from Kilombero.

12.4.3. Field and laboratory procedures

The baseline screening was carried out as follows: in early March 2009, over a period of 3 weeks, every morning between 08:00 and 09:30 hours, ~120 children were called from class and given a stool collection container labeled with unique identification numbers (IDs). Children were asked to return the container filled with a lime-sized own fresh morning stool sample the following day. Upon collection, filled stool containers were ordered by increasing IDs in a wooden transport-shelf and promptly transferred to the HCLU in Zanzibar Town.

At HCLU, duplicate Kato-Katz thick smears were prepared from each stool sample, using standard 41.7 mg templates (Katz et al., 1972). All Kato-Katz thick smears were examined quantitatively by one of four experienced laboratory technicians for hookworm eggs after a clearing time of 20-40 min in the morning and by another one of four experienced laboratory

technicians for *T. trichiura* and *Ascaris lumbricoides* eggs in the afternoon. Slides were numbered with the child's ID plus letter A or B, and each microscopist adhered to either the A or B series to avoid duplicate reading of the same stool sample by the same technician.

With regard to the FLOTAC method, ~1 g of stool, systematically obtained from each third stool sample in the transport-shelf, was weighed in a plastic tube, filled with 10 ml of 5% formaldehyde. Stool samples were suspended with a wooden spatula and stored at room temperature until further use.

Three to 9 weeks after the collection of the first stool sample, children who had their stool samples examined both by the Kato-Katz and FLOTAC techniques were invited to submit a second stool sample. Samples were again processed with duplicate Kato-Katz, and ~1 g of stool was preserved in 5% formaldehyde and stored at room temperature. Of note, children with a *T. trichiura* infection as identified by duplicate Kato-Katz thick smear readings at baseline and meeting other inclusion criteria, were treated with one of the following four drug regimens: (i) albendazole (400 mg) plus placebo; (ii) albendazole plus ivermectin (200 µg/kg); (iii) mebendazole (500 mg) plus placebo; and (iv) mebendazole plus ivermectin. Drugs were administered shortly after the end of the baseline screening in late March 2009. Follow-up stool samples from children who had received anthelmintic drugs were collected within 3-5 weeks after treatment.

The administration of albendazole and praziquantel to all school children in Kinyasini and Kilombero (and other schools) regardless of whether or not children participated in our study was carried out by members of the HCLU in late May 2009, when all available follow-up stool samples had been collected.

After completion of the trial in late May 2009, in the last 2 weeks of the study, all formaldehyde-preserved stool samples were examined with the FLOTAC basic technique (Cringoli et al., 2010). Since FLOTAC had not been implemented at HCLU before, all 22 laboratory workers, including eight microscopists, underwent a 3-day training workshop with two FLOTAC specialists from Italy to become acquainted with this new diagnostic procedure.

We used flotation solution no. 4 (FS4; sodium nitrate: NaNO₃ 315 g plus 685 ml H₂O; specific gravity (s.g.) = 1.20) in light of our preceding results for the diagnosis of soil-transmitted helminth infections (Utzinger et al., 2008; Knopp et al., 2009c; Cringoli et al., 2010). Each preserved stool suspension was pressed through a tea sieve using a wooden spatula and adding 10 ml of 0.9% NaCl. The supernatant was equally distributed in two labeled 15 ml plastic tubes and centrifuged for 3 min at 170 g in a Hettich EBA centrifuge (Tuttlingen, Germany). Subsequently, the supernatant was discarded and each tube filled to

the 6 ml mark with FS4. The pellets were suspended by pipetting the solution up and down, and 5 ml of the suspension were transferred into one of the two chambers of the labeled FLOTAC apparatus. Next, the FLOTAC apparatus was centrifuged for 5 min at 120 g in a Hettich Universal 320 centrifuge (Tuttlingen, Germany). Finally, after translation of the top portion of the FLOTAC apparatus, the observation grids of both chambers were examined for soil-transmitted helminth eggs at 100x magnification.

Fecal egg counts (FECs) for each helminth species were recorded separately for each Kato-Katz thick smear and each of the two FLOTAC observation grids. For quality control, 10% of the slides and observation grids were re-examined by a senior laboratory technician. In case the senior technician detected one or several eggs of a helminth species that had not been recorded in the original reading, the former result was considered as false-negative and replaced by the result of the senior technician. Moreover, in case of deviations in FECs of more than 10%, the original egg count was replaced by the result of the senior technician. In both cases, the microscopist was advised to read more carefully the following days. All Kato-Katz thick smears would have been re-read if there were discrepancies in the FECs in more than 20% of the re-examined slides, but this was never the case over the course of our study. The microscopists reading the FLOTAC observation grids were blinded to the results derived from the Kato-Katz method.

12.4.4. Statistical analysis

Data were entered twice in Microsoft Excel version 10.0 (2002, Microsoft Corporation; Redmond, WA, USA) and checked for consistency with EpiData version 3.1 (EpiData Association; Odense, Denmark). Discrepancies were removed by consulting original data records. Data sets for Kato-Katz and FLOTAC results from the baseline screening and follow-up were merged by ID. Statistical analyses were carried out with STATA version 10 (StataCorp.; College Station, TX, USA).

For method comparisons, only individuals whose stool samples were subjected to duplicate Kato-Katz and FLOTAC at baseline *or* follow-up were included. We used the combined results of duplicate Kato-Katz and two FLOTAC observation grids as diagnostic ‘gold’ standard. A child with egg-positive microscopic test results in any Kato-Katz thick smear or FLOTAC observation grid was considered a true-positive. We assumed 100% specificity, and hence the complete absence of false-positive results for Kato-Katz and FLOTAC on the basis of unambiguously identifiable soil-transmitted helminth eggs under a

microscope by experienced technicians. The sensitivity (proportion of true-positives detected by the test (Kelly et al., 2008)) was calculated for duplicate Kato-Katz thick smears or the FLOTAC basic technique in relation to our ‘gold’ standard. The agreement between the results of the FLOTAC basic technique and duplicate Kato-Katz thick smear readings examined at baseline and follow-up was assessed for the diagnosis of *A. lumbricoides*, hookworm, and *T. trichiura* using kappa (κ) statistics (Cohen, 1960). Interpretation of κ statistics was as follows: <0.00 indicating no agreement, 0.00-0.20 indicating poor agreement, 0.21-0.40 indicating fair agreement, 0.41-0.60 indicating moderate agreement, 0.61-0.80 indicating substantial agreement, and 0.81-1.00 indicating almost perfect agreement (Landis and Koch, 1977). The McNemar test was used to assess the inter-method differences in sensitivities, considering only individuals who were identified as positive for *A. lumbricoides*, hookworm, or *T. trichiura* according to the diagnostic ‘gold’ standard (Hawass, 1997). Statistical significance was given for *P*-values <0.05. The difference in intra-method sensitivity assessed before and after treatment was determined based on the assumption that non-overlapping 95% confidence intervals (CIs) indicate statistical significance.

Helminth-specific FECs of each individual were expressed as eggs per gram of stool (EPG), calculated by multiplying the sum of the two FECs from duplicate Kato-Katz thick smears by a factor 12. For FLOTAC, the FECs obtained from the two observation grids were added and multiplied by a factor (1 / weight of stool sample) and expressed as EPG. The geometric mean (GM) EPG of the study cohort was calculated using the normal logarithm of the EPG plus 1 ($GM = \exp((\sum \log(EPG + 1)) / n) - 1$), where $\log(EPG + 1)$ is the sum of the logarithm of each individual EPG, and one egg is added to each count to permit the calculation of the logarithm in case of EPG = 0 (Montresor et al., 1998). We calculated 95% CIs for sensitivity and the arithmetic mean (AM) EPGs and GM EPGs of the study cohort.

Participants with complete data from the baseline *and* follow-up survey, who received treatment and who were identified as positive for *A. lumbricoides*, hookworm, or *T. trichiura* according to our ‘gold’ standard at the baseline survey were included in the calculation of cure rate (CR) and egg reduction rate (ERR). The CR was determined as the percentage of children excreting eggs before treatment according to the ‘gold’ standard who became negative after treatment according to either the Kato-Katz or the FLOTAC method. CRs derived by the Kato-Katz or FLOTAC method were compared using a two-sample test of proportion. The ERR determined with Kato-Katz and FLOTAC from the treated children was calculated according to World Health Organization (WHO) guidelines (Montresor et al., 1998), as follows: $ERR = ((GM\ EPG\ before\ treatment - GM\ EPG\ after\ treatment) / GM\ EPG$

before treatment) x 100. The group GM EPG used to determine the ERR was calculated from the group of individuals identified as positive for *A. lumbricoides*, hookworm, or *T. trichiura* according to the diagnostic ‘gold’ standard at the baseline survey.

12.5. Results

12.5.1. Operational results

Consent to participate in our trial was given by the parents and guardians of 1240 children, among whom 1066 children provided a stool sample at baseline. For FLOTAC examinations, 385 (36%) stool samples were preserved in 5% formaldehyde at baseline (Figure 11, left arm). Due to inaccurate preparation, a sudden power cut, and the flotation of stool debris, which hindered subsequent microscopic examinations, and because of erroneous labeling, the results from 32 stool samples preserved at baseline were not available. Additionally, five IDs did not match the IDs from the Kato-Katz results. For another five IDs only a single instead of duplicate Kato-Katz results were available. Hence, 343 among the 385 individuals (89%) had complete FLOTAC and duplicate Kato-Katz results at baseline. Among them 182 (53%) were girls and 161 (47%) boys. The age ranged between 6 and 20 years with a median of 11 years.

A second stool sample was preserved from 288 among the 385 individuals (75%) 3-9 weeks after the collection of the first stool sample (Figure 11, right arm). Among them 204 children were given one of four different anthelmintic treatments. Results from 18 preserved stool samples were lost due to the flotation of debris or incorrect labeling. Hence, 270 preserved stool samples were examined with the FLOTAC basic technique at follow-up. Complete FLOTAC and duplicate Kato-Katz results were available from 269 (70%) individuals at follow-up.

Since 29 IDs from the first and second stool examination data set did not match, complete examination data from the baseline and follow-up survey were available from 240 among the 385 originally selected study participants (62%). Among them, 174 were given anthelmintic drugs.

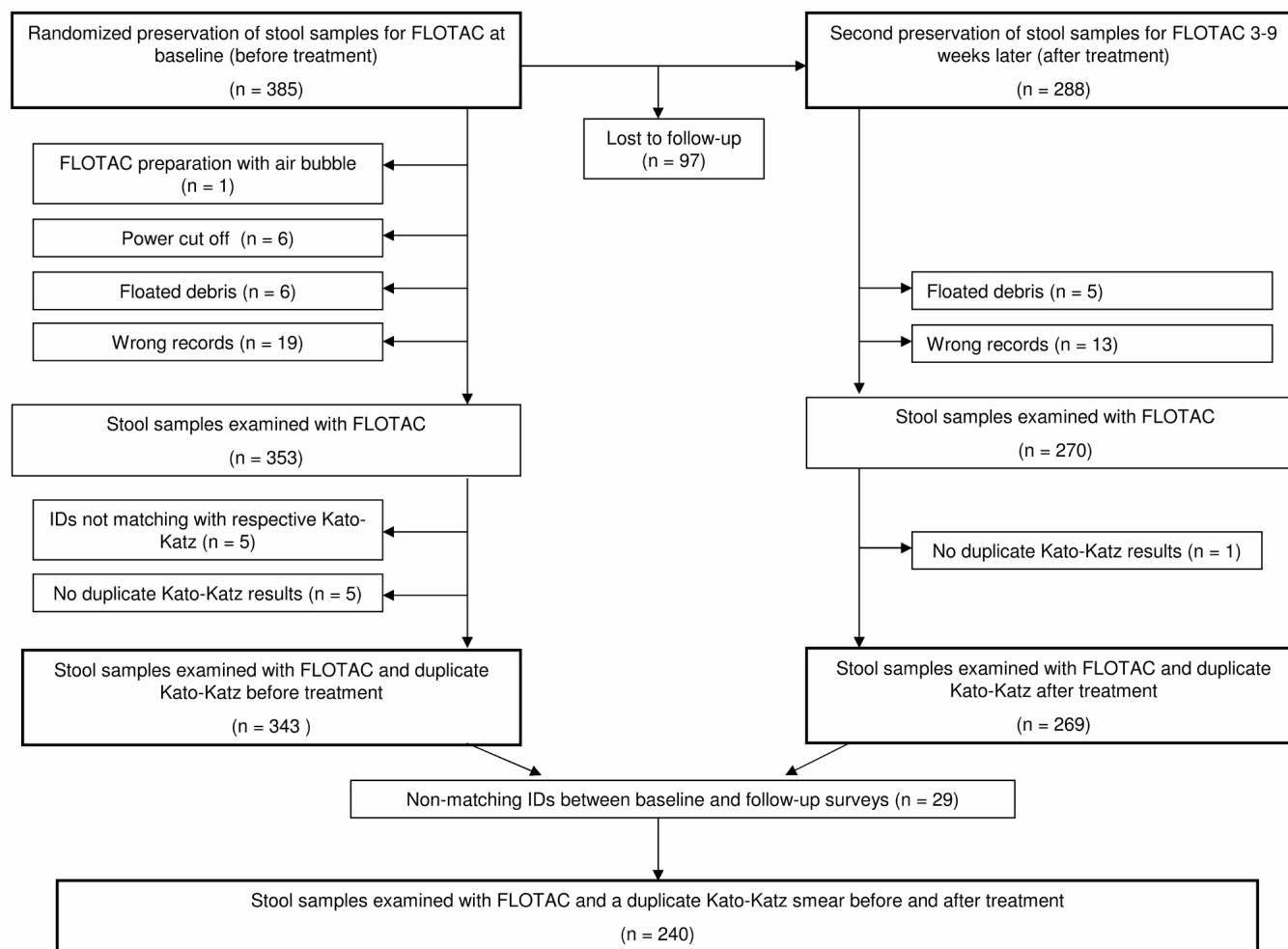


Figure 11. Number of stool samples examined with the Kato-Katz and FLOTAC method at baseline and follow-up.

Flow chart detailing the data loss during stool preservation for FLOTAC, examination, data recording and matching FLOTAC results with duplicate Kato-Katz thick smear readings within the frame of a randomized controlled trial on anthelmintic drug efficacy and safety carried out in Zanzibar in early 2009.

12.5.2. Method comparison: diagnostic sensitivity

Results presented in Table 9 and Table 10 show that the FLOTAC basic technique detected *T. trichiura* and *A. lumbricoides* infections with a higher sensitivity than duplicate Kato-Katz thick smears, but was less sensitive in detecting hookworm eggs, both at baseline and follow-up. At baseline, the sensitivity of FLOTAC for the diagnosis of *T. trichiura*, *A. lumbricoides* and hookworm was 95.0%, 87.5% and 54.1%, respectively, whereas the respective sensitivity of Kato-Katz was 88.0%, 67.5% and 81.1%. At follow-up, the sensitivity of FLOTAC for the diagnosis of *A. lumbricoides*, *T. trichiura* and hookworm was 97.4%, 93.3% and 61.2%, respectively, whereas the sensitivity of Kato-Katz was 41.7%, 84.9% and 77.6%, respectively.

The inter-method sensitivity between FLOTAC and Kato-Katz differed significantly for the detection of *T. trichiura* at baseline and follow-up ($P = 0.012$ and $P = 0.030$) and for hookworm at baseline ($P = 0.006$). The intra-method sensitivity assessed at baseline and follow-up differed significantly for the diagnosis of *A. lumbricoides* only, according to non-overlapping 95% CIs.

Moderate-to-substantial agreement between the two diagnostic techniques was observed for all helminths investigated, before and after treatment. The highest agreement ($\kappa = 0.74$) was observed for *T. trichiura* both before and after treatment, whereas the lowest agreement was noted for hookworm diagnosis at baseline ($\kappa = 0.44$).

12.5.3. Observed prevalence and infection intensities

In line with a higher sensitivity of the FLOTAC basic technique for the diagnosis of *A. lumbricoides* and *T. trichiura*, the observed prevalences of *T. trichiura* and *A. lumbricoides* infections determined with FLOTAC were higher compared to the ones derived by the Kato-Katz method. The opposite was observed for hookworm. At baseline, *T. trichiura*, hookworm and *A. lumbricoides* infections were detected in 67.1%, 11.7% and 10.2% of the children, respectively, when using FLOTAC. The respective prevalences according to Kato-Katz were 62.1%, 17.5%, and 7.9% (Table 9). Considering the results from the two methods combined, the respective prevalences were 70.6%, 21.6%, and 11.7%. The GM EPGs revealed with the Kato-Katz method were higher than those obtained with FLOTAC, showing values of 18.9 EPG for *T. trichiura*, 1.2 EPG for hookworm, and 0.9 EPG for *A. lumbricoides* vs. 9.7 EPG, 0.3 EPG and 0.6 EPG, respectively.

At follow-up, after 193 out of the 269 children (72%) had received experimental treatment, observed prevalences of *T. trichiura*, hookworm and *A. lumbricoides* infections had decreased to 57.3%, 11.2% and 4.1%, respectively, according to the FLOTAC technique. The respective prevalences according to the Kato-Katz method were 52.0%, 14.1% and 1.9% (Table 10). Results of both methods combined revealed prevalences of 61.3%, 18.2% and 4.5%, respectively. As expected, the GM EPGs were lower at follow-up than at baseline. The Kato-Katz method revealed GM EPGs of 10.5 EPG, 0.8 EPG and 0.2 EPG for *T. trichiura*, hookworm and *A. lumbricoides*, respectively. The FLOTAC method revealed respective GM EPGs of 4.4 EPG, 0.2 EPG and 0.2 EPG.

12.5.4. Estimated CR and ERR

Table 11 shows diagnostic method-specific CRs and ERRs estimated for those children who were treated, had complete data records and were identified as positive for *A. lumbricoides*, hookworm, or *T. trichiura* according to the ‘gold’ standard at the baseline survey. Employing duplicate Kato-Katz thick smears before and after treatment revealed CRs of 91.3%, 61.2% and 41.4% against *A. lumbricoides*, hookworm and *T. trichiura* infections, respectively. The estimated CRs using FLOTAC were lower for *A. lumbricoides* (82.6%) and *T. trichiura* (36.2%), but higher for hookworm (69.4%). However, none of the differences showed statistical significance. The ERRs determined with the Kato-Katz method for *A. lumbricoides*, hookworm and *T. trichiura* infections were 99.9%, 89.9% and 87.6%, respectively, and with the FLOTAC method 99.4%, 65.5% and 80.7%, respectively.

A total of 66 children had a stool sample examined with duplicate Kato-Katz thick smears and the FLOTAC basic technique at baseline and follow-up, without treatment in between. Among them, eight, seven and four children were identified to be infected with *T. trichiura*, hookworm and *A. lumbricoides*, respectively, by the Kato-Katz method at baseline (Table 12). The FLOTAC technique identified 22, five and four positive children, respectively. At follow-up, 24 and nine children were infected with *T. trichiura* and hookworm, respectively, according to the Kato-Katz method. The FLOTAC technique identified 26 children with a *T. trichiura* infection, 10 with hookworm eggs in their stool and one case of *A. lumbricoides*.

Table 9. Diagnostic accuracy of duplicate Kato-Katz thick smears and the FLOTAC basic technique at baseline.

		<i>T. trichiura</i>			Hookworm			<i>A. lumbricoides</i>		
		n pos			n pos			n pos		
		/EPG	%	95% CI	/EPG	%	95% CI	/EPG	%	95% CI
Prevalence	Kato-Katz	213	62.1	(56.9-67.3)	60	17.5	(13.5-21.5)	27	7.9	(5.0-10.7)
	FLOTAC	230	67.1	(62.1-72.1)	40	11.7	(8.3-15.1)	35	10.2	(7.0-13.4)
	‘Gold’ standard	242	70.6	(65.7-75.4)	74	21.6	(17.2-25.9)	40	11.7	(8.3-15.1)
Kato-Katz (2 slides)	Lower quartile (25%)	0			0			0		
	Median	36.0			0			0		
	Upper quartile (75%)	156.0			0			0		
	Arithmetic mean	235.2		(153.9-316.5)	43.6		(20.2-67.0)	970.4		(257.0-1683.8)
	Geometric mean	18.9		(14.2-25.2)	1.2		(0.8-1.7)	0.9		(0.5-1.3)
FLOTAC (2 chambers)	Lower quartile (25%)	0			0			0		
	Median	11.3			0			0		
	Upper quartile (75%)	60.0			0			0		
	Arithmetic mean	75.6		(47.7-103.5)	3.1		(0.2-5.9)	101.5		(10.1-193.0)
	Geometric mean	9.7		(7.6-12.3)	0.3		(0.2-0.4)	0.6		(0.3-0.9)
Sensitivity	Kato-Katz		88.0	(84.6-91.5) ^{a,b}		81.1	(76.9-85.2) ^{c,d}		67.5	(62.5-72.5) ^{e,f}
	FLOTAC		95.0	(92.7-97.3)		54.1	(48.8-59.3)		87.5	(84.0-91.0)

Prevalence, quartiles, arithmetic mean and geometric mean eggs per gram of stool (EPG), and sensitivity with 95% confidence intervals (CI), as determined from stool samples examined with duplicate Kato-Katz thick smears and the FLOTAC basic technique at baseline in 343 school children from Kinyasini and Kilombero primary schools, Zanzibar, in March 2009. The diagnostic ‘gold’ standard was derived by the combined results of duplicate Kato-Katz thick smears and the FLOTAC basic technique.

^a Differences in sensitivities determined by the McNemar test on positive individuals: $P = 0.012$

^b κ measure of agreement taking into account positive and negative individuals: $\kappa = 0.74$

^c Differences in sensitivities determined by the McNemar test on positive individuals: $P = 0.006$

^d κ measure of agreement taking into account positive and negative individuals: $\kappa = 0.44$

^e Differences in sensitivities determined by the McNemar test on positive individuals: $P = 0.098$

^f κ measure of agreement taking into account positive and negative individuals: $\kappa = 0.68$

Table 10. Diagnostic accuracy of duplicate Kato-Katz thick smears and the FLOTAC basic technique at follow-up.

		<i>T. trichiura</i>			Hookworm			<i>A. lumbricoides</i>		
		n pos			n pos			n pos		
		/EPG	%	95% CI	/EPG	%	95% CI	/EPG	%	95% CI
Prevalence	Kato-Katz	146	54.0	(48.3-60.3)	36	13.4	(9.3-17.5)	5	1.9	(0.6-4.3) ^e
	FLOTAC	154	57.3	(51.3-63.2)	30	11.2	(7.4-14.9)	11	4.1	(2.1-7.2) ^e
	‘Gold’ standard	165	61.3	(55.5-67.2)	49	18.2	(13.6-22.9)	12	4.5	(2.3-7.7) ^e
Kato-Katz	Lower quartile (25%)	0			0			0		
	Median	12.0			0			0		
	Upper quartile (75%)	90.0			0			0		
	Arithmetic mean	138.3		(90.6-186.0)	31.1		(4.0-58.1)	168.0		(0-341.5)
	Geometric mean	10.5		(7.5-14.6)	0.8		(0.5-1.2)	0.2		(0.01-0.3)
FLOTAC	Lower quartile (25%)	0			0			0		
	Median	2.7			0			0		
	Upper quartile (75%)	18.0			0			0		
	Arithmetic mean	35.6		(22.1-49.1)	1.4		(0.1-2.7)	78.2		(0-201.8)
	Geometric mean	4.4		(3.3-5.7)	0.2		(0.1-0.3)	0.2		(0.03-0.3)
Sensitivity	Kato-Katz		84.9	(80.6-89.1) ^{a,b}		77.6	(72.6-82.5) ^{c,d}		41.7	(35.8-47.6) ^{f,g}
	FLOTAC		93.3	(90.4-96.3)		61.2	(55.4-67.1)		97.4	(95.4-99.3)

Prevalence, quartiles, arithmetic mean and geometric mean eggs per gram of stool (EPG), and sensitivity with 95% confidence intervals (CI), as determined from stool samples examined with duplicate Kato-Katz thick smears and the FLOTAC basic technique at follow-up in 269 school children from Kinyasini and Kilombero primary schools, Zanzibar, in May 2009. The diagnostic ‘gold’ standard was derived by the combined results of duplicate Kato-Katz thick smears and the FLOTAC basic technique.

^a Differences in sensitivities determined by the McNemar test on positive individuals: $P = 0.030$

^b κ measure of agreement taking into account positive and negative individuals: $\kappa = 0.73$

^c Differences in sensitivities determined by the McNemar test on positive individuals: $P = 0.201$

^d κ measure of agreement taking into account positive and negative individuals: $\kappa = 0.50$

^e Binomial exact 95% confidence intervals

^f Differences in sensitivities determined by the McNemar test on positive individuals: $P = 0.417$

^g κ measure of agreement taking into account positive and negative individuals: $\kappa = 0.49$

Table 11. Drug efficacy as determined with the Kato-Katz method and FLOTAC basic technique.

		<i>T. trichiura</i>			Hookworm			<i>A. lumbricoides</i>		
		n pos			n pos			n pos		
		/EPG	%	95% CI	/EPG	%	95% CI	/EPG	%	95% CI
Baseline										
Prevalence	Kato-Katz	174	100		41	83.7	(73.0-94.4)	19	82.6	(65.9-99.4)
	FLOTAC	163	93.7	(90.0-97.3)	25	51.0	(36.5-65.5)	22	95.7	(86.6-100)
	‘Gold’ standard	174	100		49	100		23	100	
GM	Kato-Katz	121.4		(98.3-149.7)	42.6		(22.4-80.5)	1119.2		(220.7-5659.6)
	FLOTAC	29.7		(22.9-38.4)	2.0		(1.0-3.5)	193.7		(61.9-601.6)
Follow-up										
Prevalence	Kato-Katz	102	58.6	(51.2-66.0)	19	38.8	(24.6-52.9)	2	8.7	(0-21.2)
	FLOTAC	111	63.8	(56.6-71.0)	15	30.6	(17.2-44)	4	17.4	(6.3-34.2)
GM	Kato-Katz	15.1		(10.0-22.5)	4.3		(1.8-9.3)	1.2		(0-5.8)
	FLOTAC	5.7		(4.1-7.8)	0.7		(0.3-1.2)	1.1		(0-4.1)
CR	Kato-Katz		41.4	(34.1-48.7) ^a		61.2	(47.6-74.9) ^b		91.3	(79.8-100) ^c
	FLOTAC		36.2	(29.1-43.3)		69.4	(56.5-82.3)		82.6	(67.1-98.1)
ERR (GM)	Kato-Katz		87.6			89.9			99.9	
	FLOTAC		80.7			65.5			99.4	

Prevalence, geometric mean (GM) eggs per gram of stool (EPG), cure rate (CR) and egg reduction rate (ERR), as determined from stool samples examined with duplicate Kato-Katz thick smears and the FLOTAC basic technique in relation to the diagnostic ‘gold’ standard from school children treated with anthelmintic drugs. The diagnostic ‘gold’ standard was derived by the combined results of duplicate Kato-Katz thick smears and the FLOTAC basic technique.

^a Two-sample test of proportions: $P = 0.322$

^b Two-sample test of proportions: $P = 0.396$

^c Two-sample test of proportions: $P = 0.381$

Table 12. Infection characteristics in 66 untreated children at baseline and follow-up.

		<i>T. trichiura</i>			Hookworm			<i>A. lumbricoides</i>		
		n pos			n pos			n pos		
		/EPG	%	95% CI	/EPG	%	95% CI	/EPG	%	95% CI
Baseline										
Prevalence	Kato-Katz	8	12.1		7	10.6		4	6.1	
	FLOTAC	22	33.3		5	7.6		4	6.1	
GM	Kato-Katz	0.8		(0.2-1.7)	0.6		(0.1-1.2)	0.4		(0-0.9)
	FLOTAC	1.8		(0.8-3.3)	0.1		(0-0.2)	0.2		(0-0.5)
Follow-up										
Prevalence	Kato-Katz	24	36.4		9	13.6		0	0	
	FLOTAC	26	39.4		10	15.2		1	1.5	
GM	Kato-Katz	4.2		(2.0-8.2)	0.8		(0.2-1.7)	0		
	FLOTAC	2.1		(1.0-3.6)	0.3		(0.1-0.5)	0.04		(0-0.1)

Prevalence and geometric mean (GM) eggs per gram of stool (EPG), as determined from stool samples examined with duplicate Kato-Katz thick smears and the FLOTAC basic technique at baseline and follow-up from 66 school children not treated with anthelmintic drugs.

12.6. Discussion

We found a significantly higher sensitivity of the FLOTAC basic technique compared to the Kato-Katz method for the diagnosis of *T. trichiura*, both before and after anthelmintic drug administration. The sensitivity of FLOTAC was also higher for the diagnosis of *A. lumbricoides* at both time points, but the difference showed no statistical significance. With regard to hookworm diagnosis, FLOTAC showed a significantly lower sensitivity before experimental chemotherapy. The intra-method sensitivity assessed before and after treatment showed considerable heterogeneity for *A. lumbricoides* diagnosis, but not for the other two soil-transmitted helminth species investigated, notwithstanding significantly lower FECs after treatment for each species. In general, the GM EPGs obtained with the Kato-Katz method were several-fold higher than those derived from the FLOTAC method. ERRs determined by FLOTAC after anthelmintic treatment were lower than those derived by the Kato-Katz method. There was no statistically significant difference in the CRs as determined by the Kato-Katz or FLOTAC method. However, the FLOTAC revealed somewhat lower CRs than the Kato-Katz method for both *T. trichiura* (36% vs. 41%) and *A. lumbricoides* (83% vs. 91%). The opposite was found for hookworm (69% vs. 61%).

The higher sensitivity of the FLOTAC basic technique for *A. lumbricoides* and *T. trichiura* diagnosis compared to the Kato-Katz method is in line with previous studies (Knopp et al., 2009c; Glinz et al., 2010). The lower sensitivity for detecting hookworm eggs reported here, however, is in contrast to prior investigations performed with stool samples from Côte d'Ivoire and Zanzibar (Utzinger et al., 2008; Knopp et al., 2009c; Glinz et al., 2010). In our hands now, the sensitivity of FLOTAC for hookworm diagnosis was as low as 54% at baseline and slightly higher at follow-up (61%), whereas in the previous studies, sensitivities above 80% were reported (Utzinger et al., 2008; Knopp et al., 2009c). Four issues are offered for consideration, which might explain these observations. First, in the current study, we rigorously adhered to examining Kato-Katz thick smears within 20-40 min after preparation for hookworm egg counts to avoid over-clearance due to glycerol-soaked cellophane strips (Martin and Beaver, 1968). This had likely benefited the sensitivity outcome of Kato-Katz. Indeed, a limitation of our previous studies had been that we examined the slides for hookworm eggs only after 40-60 min post-preparation, which might have resulted in hookworm egg over-clearance (Utzinger et al., 2008; Knopp et al., 2009c).

Second, the stool samples in the previous studies were preserved in sodium acetate-acetic acid-formalin (SAF), whereas in the current study 5% formaldehyde was used. A potential

negative impact of the stool preservation media and FS on fragile hookworm eggs have been discussed before (Knopp et al., 2009a; Cringoli et al., 2010; Glinz et al., 2010).

Third, the higher sensitivity of FLOTAC for hookworm diagnosis at follow-up compared to baseline is pointing to a negative impact of the duration of stool preservation on hookworm eggs (samples collected at follow-up had at least a 3-week shorter preservation period than samples preserved at the baseline survey), which is in line with findings from Côte d'Ivoire (Knopp et al., 2009a; Glinz et al., 2010).

Fourth, floated organic debris might have averted the accurate detection of transparent hookworm eggs in some of our stool samples, and hence negatively impacted on the sensitivity of FLOTAC for hookworm diagnosis. This latter problem was recently observed in stool samples collected from school children in Côte d'Ivoire and Pemba island, where it was overcome by including a washing step with ether or ethyl acetate to remove the organic debris or by a higher dilution of stool samples using tap water (Rinaldi et al., 2009; Glinz et al., 2010).

The comparable sensitivities of either method at baseline and follow-up, despite a considerable decrease in FECs, suggest that a decrease in sensitivity only occurs if FECs fall under the lower detection limit of a method (i.e., 12 EPG for duplicate Kato-Katz thick smears, 24 EPG for a single Kato-Katz thick smear and 1 EPG for the FLOTAC basic technique). This suggestion is supported by the finding that those seven individuals found *A. lumbricoides*-positive by FLOTAC, but not with duplicate Kato-Katz thick smears at follow-up showed FECs of 9.8 EPGs and below, which likely explains the significant difference in the intra-method sensitivity for *A. lumbricoides* diagnosis before and after treatment.

The considerably lower numbers in the GM EPGs of our study cohort derived by FLOTAC in comparison to Kato-Katz are in line with previous studies (Utzinger et al., 2008; Knopp et al., 2009c; Glinz et al., 2010). Since there is no evidence of an upper detection limit of eggs of the FLOTAC method or of an artificial distortion in FECs associated with the smaller amount of biological material examined with the Kato-Katz method, but rather a linear relationship between FECs detected by the Kato-Katz and FLOTAC method, the following two hypotheses are offered for consideration. First, the amount of fecal material used in the Kato-Katz template (41.7 mg) is filtered, which might act like a concentration step, and hence contains a higher number of eggs (Katz et al., 1970; Perry et al., 1990), whereas the amount of stool used for FLOTAC (~1 g) is measured before filtering and contains heavy fibers, seeds and other undigested foodstuffs, but there is no concentration of

eggs. Second, the FLOTAC procedure might not bring all helminth eggs into flotation in the FS-stool suspension, but only a certain proportion. Hence, some eggs would remain in the stool debris pellet. This might happen due to a variety of reasons, including the physical damage of eggs or slightly different densities of fertilized and unfertilized eggs. Clearly, additional investigations are warranted to elucidate these hypotheses.

The somewhat lower observed CRs against *T. trichiura* and *A. lumbricoides* and the lower ERRs of all soil-transmitted helminth infections identified by FLOTAC requires further study, as it might strategically impact on future helminth control programs. For example, if one considers that anthelmintic drug efficacy is lower than generally assumed, one might conclude that preventive chemotherapy fails to bring prevalence and infection intensities to sufficiently low levels, and hence more emphasis should be placed on other preventive measures, such as health education, the implementation of sewage systems, and improving sanitation and access to clean water. Additionally, in case anthelmintic drugs are less efficacious than assumed, then the risk of resistance development is likely higher than expected, since a larger proportion of helminths survive chemotherapy, which might select for resistant strains (Doenhoff, 1998).

Before generalizing these results one must consider, however, that our study design suffers from the following shortcomings: (i) the study was not adequately powered for clinically important findings; (ii) CRs and ERRs were estimated only for individuals who were found *T. trichiura*-positive by the Kato-Katz method at baseline and occasionally co-infected with *A. lumbricoides* or hookworm; (iii) the number of individuals infected with *A. lumbricoides* or hookworm at baseline and treated with anthelmintic drugs was low; (iv) only a single stool sample was examined per person at baseline and follow-up with the FLOTAC basic technique, therefore not accounting for day-to-day variation in helminth egg output (Hall, 1981); and (v) perfect specificities were assumed for the Kato-Katz and FLOTAC method. Hence, the current sampling scheme, dictated by the primary outcome measure of the randomized controlled trial (i.e., drug efficacy against *T. trichiura*) (Knopp et al., 2010) might have biased our results and points (iv) and (v) might have resulted in an inaccurate estimate of the test sensitivity (Booth et al., 2003; Fletcher and Fletcher, 2005).

There are several causes for the high loss of samples from the baseline to the follow-up survey. First, 97 children did not submit a stool sample at follow-up. Among them 87 (89.7%) were from the *T. trichiura*-negative, and hence non-treatment group, which we did not follow as rigorously as the treated children who were part of a randomized controlled trial (Knopp et al., 2010). Second, ~1% of the stool samples could not be analyzed due to the flotation of

stool debris directly under the examination grid of the FLOTAC apparatus. Third, another 1% of the samples were lost due to a sudden power cut. Fourth, a considerable number of results was not recorded or lost due to inappropriate labeling of the tubes, the FLOTAC apparatus or the Kato-Katz slides. Points two and three constitute limitations of the FLOTAC technique that should be taken into consideration in future studies. The flotation of debris can be overcome by an additional ether washing step of the stool sample to remove organic compounds. The ether washing step results in a more clearly examinable grid of the FLOTAC apparatus and improves the detection of *A. lumbricoides* and *T. trichiura* eggs (Glinz et al., 2010). It seems, however, to impact negatively on the detection of hookworm eggs (Glinz et al., 2010), and hence a more appropriate way to lower the contamination of the FLOTAC examination grid has to be found. The problems of sudden power cuts in resource-constrained countries can be overcome by the use of mirror-operated microscopes and by hand-operated centrifuges (Jeandron et al.). Of course, these options are more laborious and time consuming, and can hence not be considered as ideal solutions for large-scale surveys. Point four implicates human failure. Since five labeling or recording steps (i.e., preservation tube, weight records, centrifugation tube, apparatus, and result records) are needed for FLOTAC, but only two (i.e., slide and result records) for Kato-Katz, the FLOTAC method is more error-prone, especially if large numbers of stool samples are processed under time constraints. In general, the application of the FLOTAC technique is more complicated and expensive than the Kato-Katz method (Speich et al., 2010). This needs to be considered when applying the FLOTAC in field laboratories and large-scale epidemiological surveys, where ease of examination is beneficial.

While it is still too early to generalize the results reported here, and the FLOTAC technique might need further optimization for reliable diagnosis of hookworm infections, this new copro-microscopic technique holds promise for simultaneous detection of the three common soil-transmitted helminths, *S. mansoni* and intestinal protozoa infections (Knopp et al., 2009c; Becker et al., 2010; Glinz et al., 2010). If these issues are solved, we are confident that the FLOTAC can serve as a viable alternative to the Kato-Katz method for anthelmintic drug efficacy assessment and for monitoring and evaluation of deworming programs, particularly in settings where infections intensities have come down to low levels after repeated treatment. The lower CRs and ERRs identified by FLOTAC warrant more investigation and, if confirmed, could strategically impact on future helminth control programs.

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12.9. Translation of abstract into German

Diagnostische Genauigkeit der Kato-Katz und FLOTAC Methode bei der Bestimmung Anthelminthischer Medikamentenwirksamkeit

Hintergrund: Diagnostische Methoden mit hoher Sensitivität werden zur genauen Bestimmung der Prävalenz und Intensität von Helminthen-Infektionen in Gegenden, in denen die Bevölkerung regelmässig entwurmt wird, und zur Überprüfung der Wirksamkeit von Medikamenten, die zur Entwurmung eingesetzt werden, benötigt. Hier vergleichen wir die diagnostische Genauigkeit der Kato-Katz Methode und der FLOTAC Technik im Rahmen einer Studie zur Wirksamkeit von Anthelminthika, die im Jahr 2009 in Sansibar, einer Insel vor Tansania, durchgeführt wurde.

Methoden/wichtigste Ergebnisse: In einer ersten Massen-Untersuchung wurden Stuhlproben von 343 Kindern mit zweifachen Kato-Katz Ausstrichen und der FLOTAC Technik untersucht. Die FLOTAC Technik hatte eine höhere Sensitivität als die Kato-Katz Methode für die Diagnose von *Trichuris trichiura* (95% vs. 88%, $p = 0.012$) und *Ascaris lumbricoides* (88% vs. 68%, $p = 0.098$), aber eine niedrigere Sensitivität für die Erkennung von Hakenwurm Eiern (54% vs. 81%, $p = 0.006$). Wenn wir die Ergebnisse beider Methoden als diagnostischen ‘Gold’ Standard zusammenfassen, waren die Prävalenzen von *T. trichiura*, Hakenwurm und *A. lumbricoides* 71% (95% Vertrauensintervall (CI): 66-75%), 22% (95% CI: 17-26%) und 12% (95% CI: 8-15%). In einer Anschlussuntersuchung, 3-5 Wochen nachdem 174 der 269 wiederholt untersuchten Kinder mit anthelminthischen Medikamenten behandelt worden waren, konnten wir Heilungsraten von 91% (95% CI: 80-100%), 61% (95% CI: 48-75%) and 41% (95% CI: 34-49%) von *A. lumbricoides*, Hakenwurm and *T. trichiura* Infektionen beobachten, wenn die Kato-Katz Methode angewendet wurde. Die FLOTAC Methode offenbarte niedrigere Heilungsraten von *A. lumbricoides* (83%, 95% CI: 67-98%) und *T. trichiura* (36%, 95% CI: 29-43%), aber eine höhere Heilungsrate von Hakenwurm Infektionen (69%, 95% CI: 57-82%). Diese Unterschiede waren statistisch nicht signifikant. Der Vergleich der beiden Methoden zeigte beachtliche Unterschiede im geometrischen Mittel der im Stuhl gezählten Helminthen Eier. Die FLOTAC Technik offenbarte eine niedrigere Eireduktionsrate nach Behandlung als die Kato-Katz Methode.

Schlussfolgerung/Bedeutung: Unsere Ergebnisse legen nahe, dass die FLOTAC Technik mit etwas Verbesserung als brauchbare Alternative zur Kato-Katz Methode in Studien zur

Wirksamkeit von Medikamenten und zur Überwachung und Evaluation von Entwurmungsprogrammen eingesetzt werden könnte. Die niedrigeren Heilungs- und Eireduktionsraten, die mit der FLOTAC Technik ermittelt wurden, sollten näher in Betracht gezogen werden und könnten einen strategischen Einfluss auf zukünftige Programme zur Kontrolle von Helminthen-Infektionen haben.

13. Spatial distribution of soil-transmitted helminths, including *Strongyloides stercoralis*, among children in Zanzibar

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13.1. Abstract

For the control of soil-transmitted helminthiasis in Zanzibar, a programme periodically distributing anthelmintic drugs to school-aged children was launched in the early 1990s. We investigated the spatial distribution of soil-transmitted helminth infections (*Ascaris lumbricoides*, hookworm and *Trichuris trichiura*), including *Strongyloides stercoralis*, in 336 children from six districts in Unguja, Zanzibar, in June 2007. One stool sample per child was examined with the Kato-Katz, Koga agar plate and Baermann methods. The point prevalence of the different helminth infections was compared to geological characteristics in the study sites. The observed prevalences for *T. trichiura*, *A. lumbricoides*, hookworm, and *S. stercoralis* were 35.5%, 12.2%, 11.9% and 2.2%, respectively, with considerable spatial heterogeneity. Whilst *T. trichiura* and hookworm infections were found in all six districts, no *A. lumbricoides* infections were recorded in the urban setting and only a low prevalence (2.2%) was observed in district South. *S. stercoralis* infections were found in four districts with the highest prevalence (4.0%) in district West. The prevalence of infection with any soil-transmitted helminth was highest in district North A (69.6%) and lowest in the urban setting (22.4%). *A. lumbricoides*, hookworm and, with the exception of district North B, *S. stercoralis* infections were observed to be more prevalent in the settings north of Zanzibar Town, which are characterized by alluvial clay soils, moist forest regions and higher precipitation. After a decade of large-scale administration of anthelmintic drugs, the prevalence of soil-transmitted helminth infections across Unguja is still considerable. Hence, additional measures, such as improving access to adequate sanitation and clean water and continued health education, are warranted to further reduce the public health importance of soil-transmitted helminthiasis in Zanzibar.

Keywords: *Ascaris lumbricoides*, hookworm, soil-transmitted helminths, *Strongyloides stercoralis*, spatial distribution, soil type, *Trichuris trichiura*, Zanzibar

13.2. Introduction

Unguja and Pemba, the two islands belonging to the Zanzibar archipelago offshore Tanzania in East Africa, are highly endemic for worm infections (Albonico et al., 1997; Mohammed et al., 2008; Stothard et al., 2008). Indeed, filaria (*Wuchereria bancrofti*), schistosomes (*Schistosoma haematobium*) and the common soil-transmitted helminths (i.e. *Ascaris lumbricoides*, hookworm and *Trichuris trichiura*) co-exist, and multiple infections of different helminth species are common (Mohammed et al., 2008; Rudge et al., 2008). Additionally, *Strongyloides stercoralis*, the most neglected of the soil-transmitted helminths (Bethony et al., 2006; Steinmann et al., 2007), is also endemic (Marti et al., 1996; Stoltzfus et al., 1997; Knopp et al., 2008).

The tropical climate of the Zanzibar archipelago and the poor hygienic conditions under which the socioeconomically deprived rural dwellers live abets the development and transmission of these helminth infections. Whilst lymphatic filariasis and soil-transmitted helminthiasis are endemic across Unguja (Mohammed et al., 2008), urinary schistosomiasis is geographically restricted. The focality of urinary schistosomiasis is governed by the distribution of its intermediate host, i.e. *Bulinus globosus* (Stothard and Rollinson, 1997). This snail species, and hence urinary schistosomiasis, is absent in the south of the island (Stothard et al., 2002). New research revealed that soil-transmitted helminth infections also show considerable spatial heterogeneity; indeed an elevated prevalence of *A. lumbricoides* and *T. trichiura* has been reported in the northern parts of Unguja (Stothard et al., 2008). One possible explanation is that the eggs of these soil-transmitted helminths remain infective for a longer period of time in the alluvium and clayey sands that are the predominant soil type in north Unguja (Stothard et al., 2008). Clayey sands can be considered as a composite matrix of coarse and fine grains where the interaction between coarser and finer grain matrices affects the overall stress-strain behaviour of the soil (Monkul and Ozden, 2007). For the transmission of hookworm, a poverty-related lifestyle, coupled with suitable environmental determinants (e.g., sandy soil type, high moisture content, suitable temperature, rainfall and sun exposure) are key factors (Brooker et al., 2004; Saathoff et al., 2005b; Hotez, 2008). The ecological and epidemiological features that might explain the spatial heterogeneity of hookworm and *S. stercoralis* distribution in Unguja, however, remain to be investigated.

Due to the high prevalence of *S. haematobium* and soil-transmitted helminth infections on Zanzibar and their negative health impacts, especially among children, a national control programme was initiated by the Zanzibar Ministry of Health in the early 1990s (Renganathan

et al., 1995). Since 1994, single-dose oral praziquantel (40 mg/kg) against schistosomiasis, and single-dose oral mebendazole (500 mg) against soil-transmitted helminthiasis were administered mainly through the existing education sector (Mohammed et al., 2008). From 2003 onwards, mebendazole has been replaced by single-dose oral albendazole (400 mg) (Stothard et al., 2008). In 2001, a programme to eliminate lymphatic filariasis was established, distributing once yearly single-dose oral albendazole (400 mg) plus ivermectin (200 µg/kg) to the whole eligible population of Unguja (Mohammed et al., 2006). Ivermectin is not only effective against filaria and *A. lumbricoides*, but is also the drug of choice to treat *S. stercoralis* infections (Gann et al., 1994), and hence is likely to have an ancillary effect against strongyloidiasis.

Despite *S. stercoralis* being endemic in Unguja and Pemba (Marti et al., 1996; Stoltzfus et al., 1997), only one of the recently published articles discussing different aspects of soil-transmitted helminthiasis focussed on *S. stercoralis* (Knopp et al., 2008). The aim of the present study was to assess the prevalence and intensity of infection of soil-transmitted helminth infections, placing particular emphasis on *S. stercoralis*, among children from selected madrassas (Koran-schools) and primary schools in the six districts of Unguja. Finally, the spatial distribution of soil-transmitted helminth infections is considered in relation to known geological features.

13.3. Materials and methods

13.3.1. Study area

The study was carried out in Unguja, the main island of Zanzibar, in June 2007. Unguja has two annual wet seasons: the Masika rains from the south lasting usually from mid-March to mid-June, and the Vuli rains from north-east occurring during November and December. The average annual rainfalls for north Unguja are 1,800 mm and for South Unguja 1500 mm (MDG-Centre, 2007). The average annual temperature in Unguja is 27°C. The pedology of Unguja soils range from alluvium to clayey sands with subordinate limestone (Kent et al., 1971). In the north-east of Unguja various soil types constitute the ground whereas at the western coastline and in the south coral limestone areas are predominant (Calton et al., 1955; Hettige, 1990).

13.3.2. Study population

Stool samples were obtained from children visiting the madrassas of five villages, namely Banda-Maji, Mahonda, Kitumba, Dole and Pete. These villages are situated in the districts North A, North B, Central, West and South, respectively, and were geographically localized using Google™-Earth 2008. All villages are quite remote, have a similar socioeconomic status with houses usually built with clay walls and thatched coconut leaves. The majority of houses have no access to the power grid.

Additionally, stool samples were collected from children visiting five peri-urban primary schools (i.e. Nyerere, Rahaleo, Fuoni, Mwenge and Regeza Mwendo) belonging to the Urban district around Zanzibar Town. All surveyed villages were located between 5 and 40 km from Zanzibar Town.

13.3.3. Field and laboratory procedures

One day before visiting the Koran-schools, the respective shehas (heads) of the communities were informed about the purpose and procedures of the study and they were asked for permission. Stool containers, a marker-pen and a pre-numbered form to fill in the name, sex and age of the children attending school were given to the teachers. Teachers were instructed to call each child attending the afternoon class, to write down his or her name on the pre-numbered list and to record the children's unique identification number on their designated stool collection containers. Containers were handed out to the children and they were invited to provide a lime-sized sample of their next morning stool and to return the filled containers.

A member of the Helminth Control Laboratory Unguja (HCLU) visited the Koran-schools early in the morning and called each child by name and compared the number on their filled container with the number on the list. This procedure was adopted to minimize the risk of sample mix-ups. The stool samples were transferred to the HCLU in Mianzini, Zanzibar Town. A similar stool collection procedure was implemented in the five urban primary schools, readily integrated in the so-called 24-school-survey, as part of an ongoing study on the epidemiology and control of soil-transmitted helminthiasis and urinary schistosomiasis (French et al., 2007).

Once the stool samples reached HCLU, they were processed promptly and examined by experienced laboratory technicians. In brief, stool samples of sufficient quantity were examined with three different methods according to the following priorities. First, a Kato-Katz thick smear was prepared using 41.7 mg templates (Katz et al., 1972). Second, a

groundnut-sized portion of each stool sample (~1 g) was subjected to the Koga agar plate method (Koga et al., 1990). Third and finally, the Baermann technique was performed (García and Bruckner, 2001). Further details pertaining to these three methods for helminth diagnosis have been presented elsewhere (Steinmann et al., 2007; Knopp et al., 2008).

Kato-Katz thick smears were examined quantitatively, i.e. the number of eggs for each helminth species was counted and recorded separately. The Koga agar plate was used to determine the presence or absence of larvae of *S. stercoralis* and hookworm. The Baermann method was used for diagnosis of *S. stercoralis* larvae. For quality control purposes, a random sample of 5% of the Kato-Katz thick smears were re-examined by a senior laboratory technician.

13.3.4. Data management and analysis

Data were double-entered in Microsoft Excel version 10.0 and checked for consistency using EpiData version 3.1 (EpiData Association; Odense, Denmark). Statistical analyses were made with STATA version 9.2 (StataCorp.; College Station, USA). The number of eggs per gram of stool (EPG) was obtained by multiplying the species-specific egg-count in a single Kato-Katz thick smear by a factor 24. We used thresholds issued by the World Health Organization (WHO) for distinguishing light, moderate and heavy infection intensities, as follows: 1-4999, 5000-49,999 and $\geq 50,000$ EPG for *A. lumbricoides*; 1-999, 1000-9999 and $\geq 10,000$ EPG for *T. trichiura*; and 1-1999, 2000-3999 and ≥ 4000 EPG for hookworm (Montresor et al., 1998). The geometric mean EPG was calculated for the children in the respective settings, using the following formula: $\exp(\sum \log(\text{EPG} + 1) / n) - 1$.

13.3.5. Ethical considerations and treatment

One part of this study was embedded in the island-wide parasitological surveys that are carried out in Unguja by the HCLU as part of their routine activities. Moreover, two schools were part of the 24-school survey that have been conducted once every year, starting in 2004, as a collaborative activity between the HCLU and the Natural History Museum (London, UK).

The institutional research commission of the Swiss Tropical Institute (Basel, Switzerland) and the institutional review board of the National Health Service Local Research Ethics Committee (application 03.36) of St. Mary's Hospital (London, UK), on behalf of the Natural

History Museum/Imperial College, approved the study. The WHO (Geneva, Switzerland), and the Ministry of Health and Social Welfare (Stone Town, Zanzibar) cleared the study protocol.

Detailed information about the study was provided to the shehas of the villages, the teachers of the Koran-schools and the headmasters of the urban primary schools. A member of the HCLU explained the study to the children. Written informed consent to all anticipated medical interventions, including parasitological surveys done at schools, was given by parents and/or legal guardians upon enrolment of their children at school. At the end of the study, children were treated regardless of their infection status with a single oral dose of albendazole (400 mg). Those children found positive for *S. stercoralis* were also treated with a single oral dose of ivermectin (200 µg/kg).

13.4. Results

13.4.1. Study compliance

In total, 336 children from the six districts of Unguja submitted one stool sample. The samples were subjected to three different methods for helminth diagnosis. Sex was recorded from 309 (92.0%) children out of which 51.8% were girls and 48.2% were boys. Age was recorded from 244 (72.6%) children. The median age was 11 years with a range from 3 to 19 years. For all remaining children, no records of age and/or sex were made available. Hookworm diagnosis was performed on 334 (99.4%) stool samples examined either with the Kato-Katz, or the Koga agar plate, or both methods. Results for *A. lumbricoides* and *T. trichiura* were available for 327 (97.3%) children who had stool samples examined with the Kato-Katz method. Finally, 319 (94.9%) stool samples were subjected both to the Baermann and the Koga agar plate method for diagnosis of *S. stercoralis*.

13.4.2. Helminth infections

Table 13 shows that helminth infections are still prevalent among children in Unguja. With regard to the three common soil-transmitted helminths, *A. lumbricoides* was the only species that was absent in one of the surveyed settings, namely in the Urban district. *S. stercoralis* was the least common helminth infection. It was found in four of the six districts with an overall prevalence of 2.2% according to combined results from the Koga agar plate and the Baermann methods. The highest prevalence (4.0%) was observed in Dole in West district.

Larval counts were usually low; the two highest counts (i.e. 4 and 9 larvae) were observed in stool samples from the two infected children in Dole in West district.

In Banda-Maji village in North A district, more than two-third of children were infected with at least one helminth species (69.6%). The second highest prevalence of helminth infection was observed in Kitumba village located in Central district (52.0%). Considerably lower prevalences were found in Mahonda village, North B district (40.0%), in Pete village, South district (39.1%), in Dole village, West district (30.8%) and, finally, in the primary schools surveyed in the Urban district (22.4%). Taken together, approximately half of the children surveyed were infected with at least one helminth (166/336, overall prevalence: 49.4%). Moreover, 47 (28.3%) children harboured two or even more helminth species concurrently.

Table 13. Prevalence (number of children positive/number of children examined) of soil-transmitted helminth infections in 336 children from the 6 districts of Zanzibar, Tanzania.

Village	District	Parasite (Method)			
		<i>A. lumbricoides</i> (K-K ^a)	<i>T. trichiura</i> (K-K ^a)	Hookworm (K-K ^a , KAP ^b)	<i>S. stercoralis</i> (BM ^c , KAP ^b)
Banda-Maji	North A	40.3 (27/67)	55.2 (37/67)	14.5 (10/69)	2.9 (2/68)
Mahonda	North B	8.3 (2/24)	29.2 (7/24)	16.0 (4/25)	0 (0/21)
Kitumba	Central	9.1 (7/77)	44.2 (34/77)	11.7 (9/77)	2.8 (2/74)
Dole	West	5.9 (3/51)	19.6 (10/51)	15.4 (8/52)	4.0 (2/50)
Pete	South	2.2 (1/46)	39.1 (18/46)	6.5 (3/46)	0 (0/39)
--	Urban	0 (0/62)	16.1 (10/62)	7.7 (5/65)	1.5 (1/67)
Total		12.2 (40/327)	35.5 (116/327)	11.9 (39/334)	2.2 (7/319)

^a K-K: Kato Katz thick smear; ^b KAP: Koga agar plate; ^c BM: Baermann

T. trichiura was the predominant helminth with an overall prevalence of 35.5% as assessed by a single Kato-Katz thick smear per child. The infection prevalence ranged from 16.1% (Urban district) to 55.2% (North A district). The large majority of infections (111/116, 95.7%) were of light intensity with EPGs below 1000 (Table 14). The remaining five children (4.3%) had a moderate infection intensity and attended the madrassas of either Pete (n = 3) or Banda-Maji (n = 2). The overall prevalence of *A. lumbricoides* infection was 12.2% according to the Kato-Katz method with the highest prevalence (40.4%) observed in Banda-Maji in North A district. Most infections (32/40, 80%) were of light intensities with EPGs below

5000. The remaining eight children (20.0%) had a moderate infection intensity and they were diagnosed either in Banda-Maji in North A district (n = 6) or in Dole in West district (n = 2). The combined results from the Kato-Katz plus the Koga agar plate methods revealed an overall hookworm prevalence of 11.9%, ranging between 6.5% (Pete, South district) and 16.0% (Mahonda, North B district). According to the quantitative Kato-Katz thick smear results, all infections were of light intensity with EPGs below 2000.

The geometric mean EPGs of the three common soil-transmitted helminths are summarised in Table 14.

Table 14. Characteristics of the three common soil-transmitted helminth infections among the 327 school-aged children from Unguja who had one stool sample quantitatively examined by the Kato-Katz method

Characteristics	Parasite		
	<i>T. trichiura</i>	<i>A. lumbricoides</i>	Hookworm
Number (%) of children infected	116 (35.5)	40 (12.2)	32 (9.8)
Infection intensity			
Light	111 (95.7%)	32 (80.0%)	32 (100%)
Moderate	5 (4.3%)	8 (20.0%)	0
Heavy	0	0	0
Geometric mean EPG (95% CI)	1326 (962-1829)	104 (86-125)	143 (98-207)

CI, confidence interval; EPG, eggs per gram of stool

13.4.3. Geological features and soil types

The geographical location of the six study sites where the study was carried out were superposed onto a Google™-Earth satellite image of Unguja (Figure 12 B) and juxtaposed to the underground composition and predominant soil types of the island (Figure 12 A), according to geological investigations published in the early 1970s (Kent et al., 1971). The geographical coordinates of the study sites are as follows: Banda-Maji (5° 57' 0" S longitude, 39° 19' 0" E latitude); Mahonda (5° 59' 0" S, 39° 15' 0" E); Kitumba (6° 07' 0" S, 39° 17' 0" E); Dole (6° 06' 0" S, 39° 14' 0" E); and Pete (6° 17' 0" S, 39° 25' 0" E). The urban area is located in Zanzibar Town (6° 09' 0" S, 39° 12' 0" E).

The underground in Banda-Maji in North A district and in Kitumba in Central district consists of fossiliferous limestone and marly sand lithology. In Mahonda in North B district and in Dole in West district, the underground is characterized by clayey sands with subordinate limestone. In Pete in South district, reefal limestone constitutes the ground. In the

urban area around Zanzibar Town, the underground consists primarily of limestone and soft sandstone or alluvium.

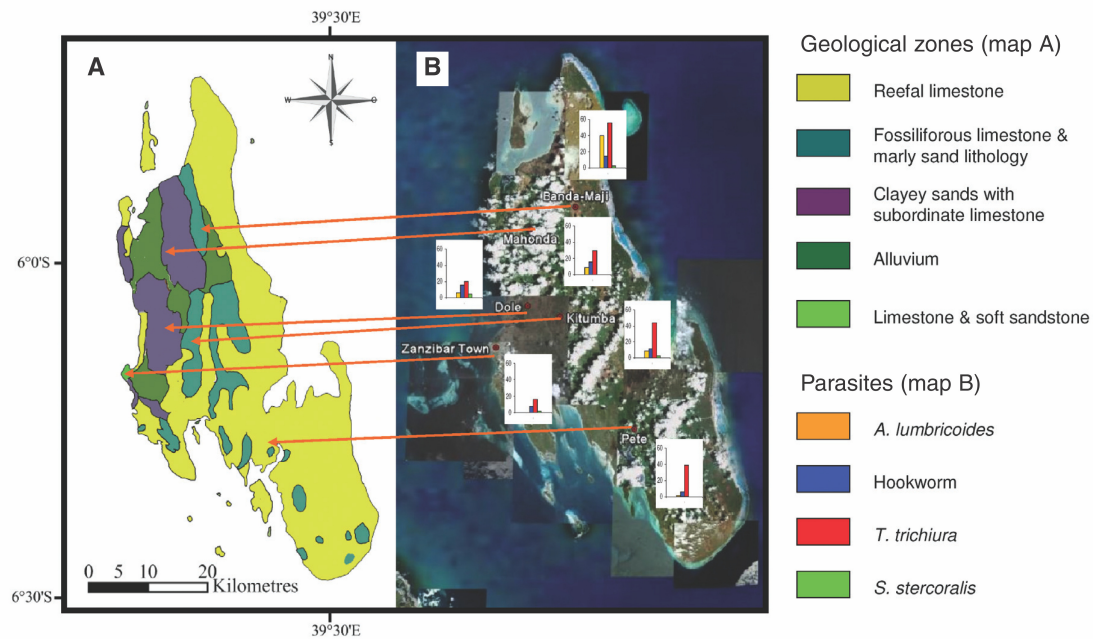


Figure 12. Map showing the geological zones of Zanzibar (adapted from Kent et al., 1971; A) and a satellite image of Zanzibar from Google™-Earth (B) indicating the surveyed settings.

13.5. Discussion

Despite considerable efforts to control helminth infections in Unguja and Pemba over the past decade (Renganathan et al., 1995; Mohammed et al., 2008), surprisingly little is known about the spatial distribution of different helminth species, including underlying demographic, environmental and socioeconomic factors. Our study sheds light on the distribution of helminth infections among children in the six districts of Unguja, five of them characterised as rural and the sixth in Zanzibar Town being urban. Emphasis is placed on the geological composition and soil types of the study locations, since these environmental factors have been shown to play an important role in the transmission of soil-transmitted helminthiasis (Brooker et al., 2004; Saathoff et al., 2005a). Particular consideration was given to *S. stercoralis* because this helminth species is often neglected in epidemiological investigations, which is partially explained by the need of particular diagnostic methods that are seldom used (Bethony et al., 2006; Steinmann et al., 2007).

In the present study we screened over 300 children from six environmentally-distinct settings and subjected a single stool sample per child to the Kato-Katz, Koga agar plate and

Baermann methods. We achieved a high compliance; more than 77% (259/336) of the children supplied stool samples of sufficient quantity to perform all three diagnostic tests. Our findings underscore the feasibility and efficiency of epidemiological studies carried out in school environments, which in turn further strengthen the collaborative links between the health and education sectors (Lengeler et al., 2002). Approximately half of the children were infected with at least one helminth species with *T. trichiura* being the predominant one. Importantly, helminth infections were primarily of light intensity according to WHO thresholds (Montresor et al., 1998), which show the ability of national helminth control programmes to reduce morbidity. Whilst *T. trichiura* and hookworm infections were observed in children from all six districts, no *A. lumbricoides* infections were diagnosed in the urban setting, and *S. stercoralis* infections were not detected in Mahonda in North B district and Pete in South district. However, the latter findings have to be interpreted with care, as the sample sizes in Mahonda and Pete were small (21 and 39 children, respectively) and only one stool sample per child was examined. Of note, *A. lumbricoides* and hookworm infections were considerably more often diagnosed in the northern part of Unguja than in the south.

We speculate that the high point-prevalence of *T. trichiura* infections in all districts (i.e. 16-55%) is, at least partially, explained by the low efficacy of the anthelmintics albendazole and ivermectin against this parasite (Marti et al., 1996; Keiser and Utzinger, 2008). The national control programme emphasising repeated administration of anthelmintic drugs to school-aged children and other high-risk groups in Unguja might therefore only have a limited effect on the transmission of trichuriasis. Moreover, it is likely that rapid re-infection occurs as long as sanitation on Unguja remains inadequate, or other treatment regimens (e.g. triple dose) or more efficacious drugs against trichuriasis have been developed and will be used in the national helminth control programme.

Distinct spatial patterns were observed for the other helminth species. From a north-south perspective (not considering the urban district in the centre), children visiting the madrassas in the northern part of the island had a higher risk to be infected with *A. lumbricoides* (odds ratio (OR) = 9.75) and hookworm (OR = 2.31) but a similar risk to be infected with *T. trichiura* (OR = 1.05) compared to the children from the madrassa in Pete, South district. However, these findings might be biased by the small sample size in Pete (n = 39) and should hence be interpreted with care. Regarding the individual study settings, the prevalence of *A. lumbricoides* in Banda-Maji in North A district (40.3%) was 4.4 to 20-fold higher than in the other districts. This observation confirms that Banda-Maji is at an elevated risk of soil-transmitted helminthiasis (Mohammed et al., 2008; Stothard et al., 2008). The high

A. lumbricoides prevalence in Banda-Maji gives rise to concerns about whether in this area school-aged children had indeed been treated regularly with anthelmintic drugs and, if so, whether our findings might be an early sign of albendazole resistance development. Interestingly, a recent study carried out in this setting reported a low cure rate (42%) when *A. lumbricoides*-infected children were given single oral dose of albendazole (Stothard et al., 2008). On the other hand, the fact that no or only very few *A. lumbricoides* infections were observed in Pete in South district and among children attending peri-urban schools might be explained by the predominant soil types (i.e. limestone and sandstone) in those areas. It is conceivable that these soil types do not provide suitable microenvironments for sustaining the longevity of *A. lumbricoides* eggs, and hence transmission of this infection is compromised. In addition, hookworm infections were less prevalent in these two settings. Indeed, the infective larval stages of hookworm require an appropriate warmth, wetness and UV protection (e.g., facilitated by vegetation coverage providing sufficient shade and moisture of the soil) for their survival, and hence transmission (Brooker et al., 2004; Saathoff et al., 2005b; Hotez, 2008). Thus, the environment in Unguja's southern part might be less suitable for their development and survival when compared to the north. The predominant geological formation in southern Unguja is based on reefal limestone with dry and sunlight-exposed savannah, whereas the areas studied north of Zanzibar Town consist of alluvium, marly and clayey sands, have higher annual precipitation and include a dense network of streams and shady forests, which provide more suitable conditions for hookworm larval development and survival. However, the absence of *A. lumbricoides* infections and the low hookworm prevalences in the Urban district are likely also a result of improved socioeconomic status leading to changes in behaviour (e.g., wearing shoes) and improved hygienic conditions, and hence a reduced risk of infection. Of note, the stool samples obtained in the Urban district were provided by children attending primary schools, which are regularly subjected to screenings and anthelmintic drug distributions conducted by the national helminth control team. The stool samples obtained from the other districts of Unguja stemmed from children visiting madrassas. These madrassas are not specifically part of the periodic school-based mass-drug administrations as children also visit public schools and anthelmintics are additionally distributed to the whole eligible island population in the frame of the programme to eliminate lymphatic filariasis. However, confounding by a lower coverage of treatment in the respective study population cannot be excluded.

The highest prevalence of *S. stercoralis* was found in Dole in West district (4.0%), but infections were absent in Mahonda, North B district and Pete, South district. Bearing in mind

that the absence of an infection might be explained by the low sample sizes there, an additional factor to note is that in Pete, the environment is drier than elsewhere, which might therefore be less suitable for the development of infective *S. stercoralis* larvae or free-living adult stages, thus precluding transmission. However, one has to keep in mind that autoinfection can perpetuate *S. stercoralis* transmission for an extended period of time (Keiser and Nutman, 2004; Vadhvani et al., 2006). It follows that this helminth is less closely linked to environmental factors than, for example, hookworm larvae. Autoinfection of *S. stercoralis* also imposes a considerable problem on copro-diagnostics. As larvae are not necessarily excreted in the stool, it is exceedingly difficult to identify infected individuals. Our recent work focussing on the diagnosis of *S. stercoralis* among schoolchildren in Unguja showed that the observed prevalence of this worm is more than double when three instead of a single stool sample were examined with a combination of the Koga agar plate and Baermann method (Knopp et al., 2008). Hence, the 'true' overall prevalence of *S. stercoralis* in the present study might well be 4% or even higher.

We conclude that soil-transmitted helminth infections are still prevalent across Unguja, but infection intensities are generally low, demonstrating the impact of the national control programme on morbidity. *T. trichiura* infections seem to be hardest to reduce with the current single-dose anthelmintic treatment campaigns. Although the observed prevalence of *S. stercoralis* was low, the 'true' prevalence might be considerably higher were more sensitive diagnostic approaches employed, and hence this parasite should not be neglected. Our findings call for continuation of the national control programme especially in the rural areas, with chemotherapy being complemented by improved access to clean water and adequate sanitation and sound health education.

13.6. Acknowledgements

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14. Patterns and risk factors of helminth infections and anemia in a rural and a peri-urban community in Zanzibar, in the context of helminth control programs

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14.1. Abstract

Background: The control of helminth infections and prevention of anemia in developing countries are of considerable public health importance. The purpose of this study was to determine patterns and risk factors of helminth infections and anemia in a rural and a peri-urban community of Zanzibar, Tanzania, in the context of national helminth control programs.

Methodology/Principal Findings: We carried out a community-based cross-sectional study in 454 individuals by examining at least two stool samples with different methods for soil-transmitted helminths (*Ascaris lumbricoides*, hookworm, *Strongyloides stercoralis*, and *Trichuris trichiura*) and one urine sample for *Schistosoma haematobium*. Finger-prick blood was taken to estimate anemia levels and to detect antibody reactions against ascariasis, strongyloidiasis and schistosomiasis, using an enzyme-linked immunosorbent assay (ELISA) approach. Parasitological methods determined a helminth prevalence of 73.7% in the rural, and 48.9% in the peri-urban setting. Most helminth infections were of light intensity with school-aged children showing the highest intensities. Multiple helminth species infections were pervasive in rural dwellers regardless of age. More than half of the participants were anemic, with a particularly high prevalence in the peri-urban setting (64.7%). Risk factors for helminth infections were age, sex, consumption of raw vegetables or salad, recent travel history, and socio-economic status.

Conclusion/Significance: After several years of chemotherapy-based morbidity control efforts in Zanzibar, helminth prevalences are still high and anemia is common, but helminth infection intensities are low. Hence, chemotherapy should be continued, and complemented with improved access to clean water, adequate sanitation, and health education, along with poverty alleviation measures for a more enduring impact.

14.2. Author Summary

In many parts of the developing world, parasitic worms and anemia are of considerable public health and economic importance. We studied the patterns and risk factors of parasitic worm infections in a rural and a peri-urban community on Zanzibar Island, Tanzania, in the context of national deworming programs. We invited 658 individuals aged between 5 and 100 years and examined their stool and urine for the presence of parasitic worm eggs. Additionally, we obtained a finger-prick blood sample to estimate the level of anemia and to assess for specific immune reactions against parasitic worm infections. We found that, despite large-scale deworming efforts in Zanzibar over the past 15 years, three-quarter of the rural participants and half of the peri-urban residents were infected with parasitic worms. Every second participant was anemic. Risk factors for a parasitic worm infection were age, sex, consumption of raw vegetables or salad, recent travel history, and socio-economic status. For a sustainable control of parasitic worm infections and prevention of anemia, access to safe and efficacious drugs, complemented with health education and improvements in water supply and adequate sanitation are necessary.

Keywords: Soil-transmitted helminthiasis, *Ascaris lumbricoides*, hookworm, *Strongyloides stercoralis*, *Trichuris trichiura*, *Schistosoma haematobium*, anemia, Zanzibar, Tanzania

14.3. Introduction

Soil-transmitted helminthiasis and schistosomiasis affect hundreds of millions of people and the global burden due to these parasitic worms might exceed 40 million disability-adjusted life years (DALYs) lost annually (Bethony et al., 2006; Hotez et al., 2006b; Steinmann et al., 2006). Chronic infections can result in negative birth outcomes, delayed physical and cognitive development during childhood, and reduced agricultural productivity among adults (WHO, 2006a; Hotez, 2009). There is growing evidence that, besides malaria and nutritional deficiencies, heavy helminth infections as well as multiple helminth species infections of light intensities, are associated with anemia (Dreyfuss et al., 2000; Hall et al., 2001; Ezeamama et al., 2005).

The global strategy for the control of soil-transmitted helminthiasis and schistosomiasis is morbidity control using single-dose orally-active anthelmintic drugs. In 2001, at the 54th World Health Assembly (WHA), member states were urged to achieve a minimum target of regular deworming of at least 75% and up to 100% of school-aged children and other groups at risk of morbidity by 2010 (WHO, 2002).

In Zanzibar, infections with soil-transmitted helminths (*Ascaris lumbricoides*, hookworm, and *Trichuris trichiura*) are highly endemic (Booth et al., 1998; Knopp et al., 2008b; Stothard et al., 2009a). *Strongyloides stercoralis* infections also occur, but few epidemiological investigations have focused on this helminth (Marti et al., 1996; Knopp et al., 2008b). With regard to schistosomiasis, *Schistosoma haematobium* is the only species endemic in Zanzibar. Its distribution is focal, linked to the distribution of the intermediate host snail (Stothard et al., 2002; Stothard et al., 2009a). Due to excessively high helminth prevalences (>90%) in school-aged children in Zanzibar, large-scale school-based administration of anthelmintic drugs (mebendazole or albendazole) was initiated in the mid-1990s (Renganathan et al., 1995), and contingent upon drug donations deworming has been carried out annually. Of note, in 2006, Zanzibar achieved the minimum target of regular administration of anthelmintic drugs to at least 75% of all school-aged children (WHO, 2008c). In addition, within the global program to eliminate lymphatic filariasis (GPELF), ivermectin plus albendazole were distributed to the entire at-risk population (excluding children younger than 5 years and pregnant women) in Zanzibar from 2001 to 2006, and a mean annual coverage rate of 80% was reached (Mohammed et al., 2006; Mohammed et al., 2008; WHO, 2008b). Importantly, ivermectin is efficacious against *S. stercoralis* (Marti et al., 1996) and albendazole against common soil-transmitted helminths (Keiser and Utzinger, 2008).

Large-scale deworming programs in Zanzibar have reduced helminth-related morbidity and are likely to have lowered overall transmission (Mohammed et al., 2008; Knopp et al., 2009). Hence, as original programmatic targets are being met, it is interesting to study the patterns and risk factors of helminth infections in contemporary times. The aim of our cross-sectional study was to assess the current prevalence and intensity of helminth infections and to determine anemia levels in different age groups in a rural and a peri-urban community in Zanzibar, in the context of helminth control programs. We used standardized, quality-controlled parasitological and serological tests, administered a questionnaire to investigate behavioral, demographic, and socio-economic risk factors for helminth infection and anemia, and determined self-reported morbidity signs that might be associated with helminth infections. Combined, this information will provide an important insight into the impact of ongoing interventions and hopefully evidence-based realignment of disease-control priorities.

14.4. Materials and methods

14.4.1. Ethics statement

The study protocol was approved by the institutional research commission of the Swiss Tropical and Public Health Institute (Basel, Switzerland). Ethical clearance was obtained from the Ethics Committee of the Ministry of Health and Social Welfare (MoHSW) in Zanzibar (application number 16). The shehas (community leaders/heads) and sub-shehas of each shehia (administrative areas) were informed about the purpose and procedures of the study. The inhabitants of the shehia were subsequently informed by the shehas. All adult participants and the parents/legal guardians on behalf of their children (aged 5-16 years) signed a written informed consent sheet. All participants were free to withdraw from the study at any time without further obligation. At the end of the study all participants were invited to learn about their parasitological results and were treated with albendazole (single 400 mg oral dose) if infected with *A. lumbricoides*, *T. trichiura* and/or hookworm, with ivermectin (single 200 µg/kg oral dose) if infected with *S. stercoralis*, and with praziquantel (single 40 mg/kg oral dose) if infected with *S. haematobium*.

14.4.2. Study setting

The Zanzibar archipelago consists of the two main islands of Unguja and Pemba with ~1 million inhabitants according to the population census in 2002. Fishing and farming are the most important economic activities. Islam is the predominant religion.

Our community-based cross-sectional study was carried out in two shehias of Unguja, in collaboration with the Helminth Control Laboratory Unguja (HCLU) of the MoHSW in June and July 2008. Bandamaji is a rural shehia, located in District North A, ~40 km from Zanzibar Town. According to the 2002 census its population consisted of 993 inhabitants and the annual growth rate in District North A is 2.4% (Central_Census_Office, 2004a). Dole is a peri-urban shehia in District West, located ~10 km north-west from Zanzibar Town. There were 2,496 inhabitants in 2002. The annual growth rate of District West is 9.2% (Central_Census_Office, 2004b). Both sites received single annual treatments of albendazole and ivermectin in the frame of the GPELF from 2001-2006, and albendazole or mebendazole plus praziquantel via the school-based deworming program till 2006. Of note, the school in Bandamaji was only opened in 2005.

14.4.3. Study participants

According to guidelines put forth by the World Health Organization (WHO), a sample size of 250 complying individuals in a geographically distinct community is needed to assess the prevalence and intensity of soil-transmitted helminth infections (Lwanga and Lemeshow, 1991; Montresor et al., 1998). Allowing for drop-out and non-compliance of 20-30%, we aimed at enrolling approximately 330 individuals per study setting, with a quarter being school-aged children (5-15 years) and the remaining three-quarter being adolescents and adults (>15 years). Hence, all individuals from Bandamaji and Dole aged 5 years and above were eligible for the study.

The shehas were asked to invite ~300 adult community members to attend an information meeting and to bring along their children. After the meeting, adolescents and adults who participated in the meeting were invited to sign an informed consent form. Additionally, children on the spot and their parents/legal guardians were asked for the children's age and whether they were interested to participate in the study. Subsequently, the first ~50 girls and the first ~50 boys aged 5-15 years, who lined up to receive a consent form and were accompanied by their parents or legal guardians who signed the form were enrolled in the study.

14.4.4. Field procedures

The shehas of both communities were consulted about the purpose of the study. After obtaining their oral consent the shehas participated actively in deciding how, when and where the specific parts of the study (e.g., information of participants, collection of stool, urine, and blood, and day for questionnaire survey) should take place. After a further meeting where the aims of the study were explained in lay terms in the local language Kiswahili to ~300 invited adult community members and participating children, written informed consent was obtained and three stool containers labeled with unique identifiers and the intended collection day were distributed. Our goal was to obtain three stool samples over consecutive days. Stool samples were collected between 08:00 and 10:00 hours by fieldworkers at a public spot and transferred to the HCLU in Zanzibar Town.

On Friday, when people tend to stay near their village for prayer, additional field procedures were carried out. Participants were interviewed with a pre-tested questionnaire about risk factors and morbidity signs that might be related to helminth infections and anemia, and about household characteristics and asset ownership (Raso et al., 2006). Subsequently, finger-prick blood was collected from each participant and the hemoglobin (Hb) level was determined with a portable Hemocue device (HemoCue Hb 201+; Sheffield, United Kingdom). Finger-prick blood samples were collected in small tubes (BD Microtainer, Ref.: 365967) and put on ice after clotting. Finally, participants were invited to submit a urine sample, which was collected between 10:00 and 14:00 hours.

14.4.5. Laboratory procedures*Parasitological examinations*

Stool and urine samples were collected to screen for helminth eggs using standardized, quality-controlled parasitological tests. All stool samples were examined at HCLU using the Kato-Katz (K-K) (Katz et al., 1972), the Baermann (BM) (García and Bruckner, 2001), and the Koga agar plate (KAP) method (Koga et al., 1991). Details of these methods have been provided elsewhere (Knopp et al., 2008a). In brief, 41.7 mg K-K thick smears were read under a microscope within 20-40 min to avoid rapid over-clearance of hookworm eggs in glycerol (Martin and Beaver, 1968). The number of *A. lumbricoides*, hookworm, and *T. trichiura* eggs were counted and recorded separately. Regarding the BM method, ~5 g of stool was put on medical gauze within a glass funnel that was filled with water and exposed to an electric light source from below. Phototactic larvae were collected after 2 hours of exposure and visualized

on microscope slides. The number of *S. stercoralis* larvae was recorded for each participant. Finally, regarding KAP, ~2 g of stool was exposed in the middle of an agar plate, and the plates were incubated for 48 hours in a humid chamber before visual examination for traces of *S. stercoralis* and/or hookworm larvae. Subsequently, the plates were washed with 10% formaldehyde and the sediment was qualitatively examined for *S. stercoralis* and hookworm larvae under a microscope.

For quality control, a senior laboratory technician re-examined a random sample of 10% of the K-K thick smears daily. If false-negatives were identified or if differences in egg counts were observed that were judged unacceptable by the study coordinator (S.K.), the respective microscopist was advised to read more carefully the next day and original results were replaced by the results of the senior technician. If there were differences judged unacceptable in more than 20% of slides subjected to quality control, all slides of the day were re-examined.

Eggs of *S. haematobium* were counted under a microscope after filtering 10 ml of vigorously shaken urine with a syringe using a 12 µm polycarbonate millipore filter (Millipore; Billerica, MA, USA) (WHO, 1991).

Serological examinations

Serum was collected to screen for antibodies against *Ascaris*, *Strongyloides*, and *Schistosoma* antigens using commercially available ELISA kits (Microwell Serum ELISA, IVD Research Inc.; Carlsbad, CA, United States of America). At HCLU, the clotted finger-prick blood samples were centrifuged at 6,000 g for 10 min in a benchtop microcentrifuge (Eppendorf Mini Spin; Hamburg, Germany). The sera were transferred into labeled Eppendorf tubes and stored at -20°C pending further analyses. For ELISA, sera were prepared following the manufacturer's manual. In brief, freshly thawed sera were diluted 1:100, 1:64 and 1:40 in dilution buffers for *A. lumbricoides*, *S. stercoralis*, and *S. haematobium*-specific ELISAs, respectively. Next, 100 µl of each serum dilution was transferred into one well of three microtiter plates coated by the manufacturer with *Ascaris*, *Strongyloides*, and *Schistosoma* antigens, respectively. At least one positive and one negative control provided by the manufacturer were included in each test. After steps of incubation, washing, adding the enzyme conjugate (protein A-peroxidase) and the chromogen tetramethylbenzidine substrate and finally stopping the reaction with 1 M phosphoric acid, the plates were read at an optical density (OD) of 450 nm using an ELISA reader (Multiskan Bichromatic, version 1.06 P; Vienna, VA, United States of America) according to the manufacturer's instructions.

14.4.6. Questionnaire survey

Participants were interviewed in Kiswahili with a pre-tested questionnaire by trained field enumerators of HCLU. The questionnaire included demographic and a series of housing characteristics (i.e., building type of walls, floor, and roof) and asset ownership (i.e., mobile phone, radio, black and white television (TV), color TV, satellite dish, video compact disc player, fan, refrigerator, bicycle, motorbike, car, stove type (electric, coal, wood), soap, access to the power grid, and animals (cattle, cow, goat, sheep, and donkey)). Risk factors for a helminth infection were determined via source of drinking water used (i.e., tap, shallow well, deep well, spring, and river), presence and type of toilet at home (no toilet – using “the bush”, latrine, or flush toilet), hand washing behavior (whether or not hands are washed with or without soap before eating and after defecation), consumption of raw vegetables or salad, consumption of unpeeled fruits, consumption of soil, always wearing shoes, sleeping under a bed net, traveling within the last two weeks, and ownership of a dog or a cat. Finally, using a recall period of two weeks, the questionnaire included 12 morbidity signs (i.e., fever, fatigue, stomach ache, diarrhea, blood in stool, blood in urine, pain when urinating, vomiting, cough, blood in sputum, itching, and headache) and six diseases (i.e., malaria, soil-transmitted helminthiasis, schistosomiasis, skin disease, eye disease, and cold).

14.4.7. Data management and statistical analysis

Parasitological and serological data were entered twice in Microsoft Excel version 10.0 (2002 Microsoft Corporation). Questionnaire data were entered twice in EpiInfo version 3.5.1 (Centers for Disease Control and Prevention; Atlanta, GA, United States of America). Double-entered datasets were compared using EpiInfo and discrepancies removed against the original records. Data were analyzed using STATA version 9.2 (StataCorp.; College Station, TX, United States of America) and R version 2.10.1 (R Development Core Team; Vienna, Austria). Only individuals who submitted at least two stool samples were included in the final analyses.

Age was stratified into four groups, (i) 5-11 years, (ii) 12-14 years, (iii) 15-59 years, and (iv) ≥ 60 years, as suggested by WHO (WHO, 2001, , 2008a). For each individual, the arithmetic mean of the helminth species-specific egg counts from the K-K thick smears was calculated and multiplied by a factor 24 to obtain a standardized measure of infection

intensity, expressed as eggs per gram of stool (EPG). Infection intensities were classified into light, moderate, and heavy, according to thresholds put forward by WHO (WHO, 2002). The lower limits of moderate and heavy infections were 5,000 and 50,000 EPG for *A. lumbricoides*, 1,000 and 10,000 EPG for *T. trichiura*, and 2,000 and 4,000 EPG for hookworm, respectively. *S. haematobium* egg counts were classified into light (1-49 eggs/10 ml of urine) and heavy (≥ 50 eggs/10 ml of urine) (WHO, 2002).

Differences in the median EPG of the four age groups were determined using the Kruskal-Wallis test. Pair-wise comparisons between the median EPG of two age groups were adjusted for multiple testing as suggested by Siegel and Castellan (Siegel and Castellan, 1988). Statistical significance was given at a p-value of 0.05. Hb thresholds used to define anemia were 115 g/l for children of both sexes aged 5-11 years, 120 g/l for children of both sexes aged 12-14 years, 120 g/l for women aged ≥ 15 years (non-pregnant) and 130 g/l for men aged ≥ 15 years, following WHO thresholds (WHO, 2001). Anemia was classified into 'moderate to severe anemia' and 'heavy anemia' when Hb values were < 90 g/l and < 70 g/l, respectively (Stolfzfus and Dreyfuss, 1998).

Antibody reactions were regarded as positive when the absorbance readings were > 0.1 OD units for *A. lumbricoides*, > 0.2 OD units for *S. stercoralis*, and ≥ 0.2 OD units for *S. haematobium*, following the manufacturer's handbook. Sensitivity (i.e., proportion of true-positives identified as positive) and specificity (i.e., proportion of true-negatives identified as negative) of the ELISA tests were determined considering the pooled results of the respective ELISA and at least two K-K this smear readings (for *A. lumbricoides*), one urine filtration (for *S. haematobium*), or at least two KAP and/or BM (for *S. stercoralis*) as diagnostic 'gold' standard. Hence, a person was considered positive for a particular parasite if at least one diagnostic test revealed a positive result.

The socio-economic status was determined according to a wealth index, calculated on the basis of housing characteristics and asset ownership. Using principal component analysis (PCA), based on the sum of household and asset characteristic scores, all interviewed participants were grouped into wealth quintiles: (i) most poor, (ii) very poor, (iii) poor, (iv) less poor, and (v) least poor (Filmer and Pritchett, 2001; Raso et al., 2005). Multivariable logistic regression was used for estimating odds ratios (ORs), including 95% confidence intervals (CIs), to determine risk factors for helminth infections and anemia, and to determine associations between helminth infections and anemia and self-reported morbidity signs and diseases, as assessed by the questionnaire. Outcomes were defined as specific helminth infection, determined by any parasitological method in at least one stool or urine sample, or

the presence of anemia, or any self-reported morbidity sign or disease. Candidate explanatory variables for the multivariable logistic regression were those which were reasonable and present in at least 5% of the interviewed participants in each community. A backward stepwise multivariable logistic regression, allowing for possible clustering within houses and stratified by community, and removing non-predicting covariates up to a significance level of 0.2 was performed using the sandwich estimator robust cluster option in STATA. The remaining variables were included into the final models and the Wald test was used to determine whether each independent variable was significantly related to the outcome variable.

14.5. Results

14.5.1. Study cohort and compliance

From 658 individuals who registered for the study and signed a written informed consent sheet, 137 (20.8%) never submitted any stool sample. A single stool sample was provided by 67 (10.2%) participants. The remaining 454 individuals (69.0%) submitted two or three stool samples, and hence were included for further analyses (Figure 13). Among them, 294 were female (64.8%) and 160 were male (35.2%). There were 270 people from Bandamaji (59.5%) and 184 from Dole (40.5%). The age groups of 5-11, 12-14, 15-59 and ≥ 60 years consisted of 106, 74, 231 and 43 individuals, respectively. The median age was 19.5 years.

Due to insufficient quantities of feces and the priority for the sequence of tests employed, 446 (98.2%), 437 (96.3%) and 411 (90.5%) individuals had at least two stool samples examined with the K-K, KAP, and BM method, respectively. Since for hookworm diagnosis the results of both K-K and KAP, and for *S. stercoralis* diagnosis the results of both KAP and BM were combined, the respective analyses included 450 (99.1%) and 443 (97.6%) individuals. Urine samples for *S. haematobium* examination were available from 351 individuals (77.3%). Finger-prick blood for estimating Hb levels was available from 352 participants (77.5%). Antibody reactions against *S. haematobium*, *S. stercoralis*, and *A. lumbricoides*, were tested using ELISA from 339 (74.7%), 337 (74.2%), and 227 (50.0%) participants, respectively. The questionnaire was completed by 375 out of the 454 individuals who submitted at least two stool samples (82.6%).

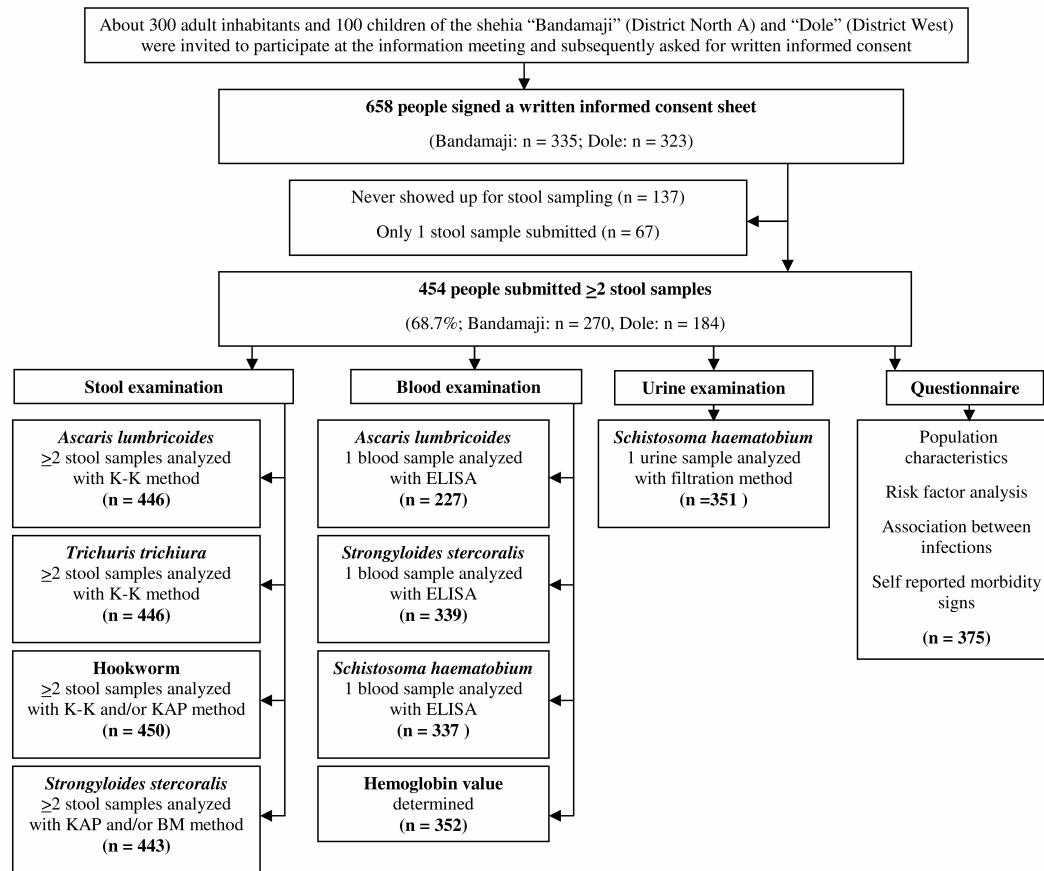


Figure 13. Study participation and compliance. Diagram detailing the study participation and compliance of community members from rural Bandamaji and peri-urban Dole, Zanzibar, in June/July 2008. All individuals providing at least two stool samples were included in the final analyses. K-K: Kato-Katz method, KAP: Koga agar plate method, BM: Baermann method.

14.5.2. Population profile

Key population characteristics as determined by the questionnaire survey, stratified by study settings (rural Bandamaji and peri-urban Dole), are presented in Table 15. Both populations differed significantly as for place of birth, place of residence, religion, educational attainment, profession, and socio-economic status. In summary, most of the rural dwellers were born in Unguja and most adolescents/adults (≥ 16 years) resided in Bandamaji for more than 10 years. They were all Muslim and farming was their primary occupation. Only 9.8% of the participants belonged to the least poor wealth quintile.

In contrast, more than a third of the peri-urban dwellers were born outside Unguja and almost a quarter of the interviewed adolescents/adults had lived in Dole for less than 10 years.

Islam is the predominant religion, but there were also Christians (15.1%). The range of occupations in Dole was broader than in Bandamaji, including a higher percentage of traders, teachers, and civil servants. More than a third of the peri-urban inhabitants belonged to the least poor wealth quintile.

Table 15. Population characteristics, according to questionnaire survey, stratified by study setting (rural Bandamaji: n = 236, peri-urban Dole: n = 139) in Zanzibar, June/July 2008.

Population characteristics	Bandamaji		Dole		Difference	
	n	%	n	%	χ^2	P-value
Place of birth						
Unguja	233	98.7	87	62.6		
Pemba	1	0.4	8	5.8		
Tanzania mainland	2	0.9	43	30.9		
Not known	0	0	1	0.7	91.4	<0.001
Residency in village^a						
<2 years	2	1.4	5	6.4		
2-5 years	7	5.0	5	6.4		
6-10 years	10	7.1	7	9.0		
>10 years	119	84.4	60	76.9		
Not applicable	3	2.1	1	1.3	4.9	0.300
Religion						
Islam	236	100	116	83.5		
Christian	0	0	21	15.1		
Other	0	0	2	1.4	41.6	<0.001
Occupation						
Preschool child	6	2.5	14	10.1		
School child	98	41.5	51	36.7		
Farmer	110	46.6	49	35.3		
Trader	2	0.9	5	3.6		
Teacher/civil servant	4	1.7	6	4.3		
Other	1	0.4	5	3.6		
Not known	15	6.4	9	6.5	23.8	0.001
Educational attainment^b						
Illiterate	65	49.2	23	31.1		
Pre school	1	0.8	1	1.4		
Primary school	51	38.6	26	35.1		
Secondary school	13	9.9	20	27.0		
Middle school/apprenticing	2	1.5	3	4.1		
Junior college or university	0	0	1	1.4	15.8	0.008
Socio-economic status						
Most poor	56	23.7	19	13.7		
Very poor	62	26.3	13	9.4		
Poor	50	21.2	25	18.0		
Less poor	45	19.1	31	22.3		
Least poor	23	9.8	51	36.7	50.0	<0.001

^aConsidered only individuals aged ≥ 16 years (Bandamaji: n = 141, Dole: n = 78).

^bConsidered only individuals who were not preschool or school children, according to occupation (Bandamaji: n = 132, Dole: n = 74).

14.5.3. Helminth infections and anemia, stratified by study setting

The overall prevalence of infection with any helminth species, according to different tests examined under a microscope was 73.7% in Bandamaji and 48.9% in Dole. In Bandamaji, the prevalence of *A. lumbricoides*, *T. trichiura*, hookworm, *S. stercoralis*, and *S. haematobium* was 49.4%, 48.7%, 31.1%, 10.3%, and 5.4%, respectively. In Dole, hookworm was the predominant species (32.2%). Infections with *A. lumbricoides* were rare (3.4%), whereas prevalences of 16.8%, 12.7%, and 11.7% were found for *T. trichiura*, *S. stercoralis*, and *S. haematobium*.

Among the infected individuals, 87.0% (120/138) and 13.0% (18/138) had a light and moderate infection with *A. lumbricoides*, 98.7% (158/160) and 1.3% (2/160) had a light and moderate infection with *T. trichiura* and, with the exception of one moderate hookworm infection, all others 99.1% (105/106) were of light intensity. No heavy infections were found for any soil-transmitted helminth. All moderate infection intensities with both *A. lumbricoides* and *T. trichiura* were observed in Bandamaji, whereas the moderate hookworm infection was found in Dole. Among the 27 *S. haematobium* infections, 22.2% (four individuals from Dole and two from Bandamaji) were heavily infected.

The prevalence of anemia was 64.7% in Dole and 50.9% in Bandamaji, with 8.1% (16/198) individuals showing moderate-to-severe anemia, and 2.5% (5/198) individuals being severely anemic.

14.5.4. Helminth infections and anemia, stratified by age group

The species-specific prevalence of helminth infections in each of the four age groups in Bandamaji and Dole is presented in Figure 14. In both settings, the prevalence of *A. lumbricoides* and *T. trichiura* decreased with age. Whilst a decrease with age was also observed for hookworm and *S. stercoralis* in Bandamaji, the age-prevalence curve for these two helminths was relatively stable in Dole. Regardless of the study setting, *S. haematobium* infections were most prevalent in the 12-14-year-old age group (17.1% in Bandamaji and 26.3% in Dole). No *S. haematobium* infections were found in the elderly (≥ 60 years). Finally, anemia peaked in the age group 12-14 years (60.6% in Bandamaji, 73.7% in Dole).

Figure 15 shows that patterns of polyparasitism differed according to setting and age. In rural Bandamaji, polyparasitism was highest in the youngest age group (5-11 years), with 36.4%, 10.9%, and 1.8% of the children concurrently infected with 3, 4, or even 5 helminth species, respectively. Polyparasitism decreased with age: 47.6% of the elderly (≥ 60 years)

were free of infection. In peri-urban Dole, concomitant multiple helminth species infections were less common. Approximately half of the participants were free of any helminth infection, and concurrent infections with 3 or 4 helminths occurred in <5% of the participants of any age group. No individual was found to be parasitized with all 5 helminths concurrently.

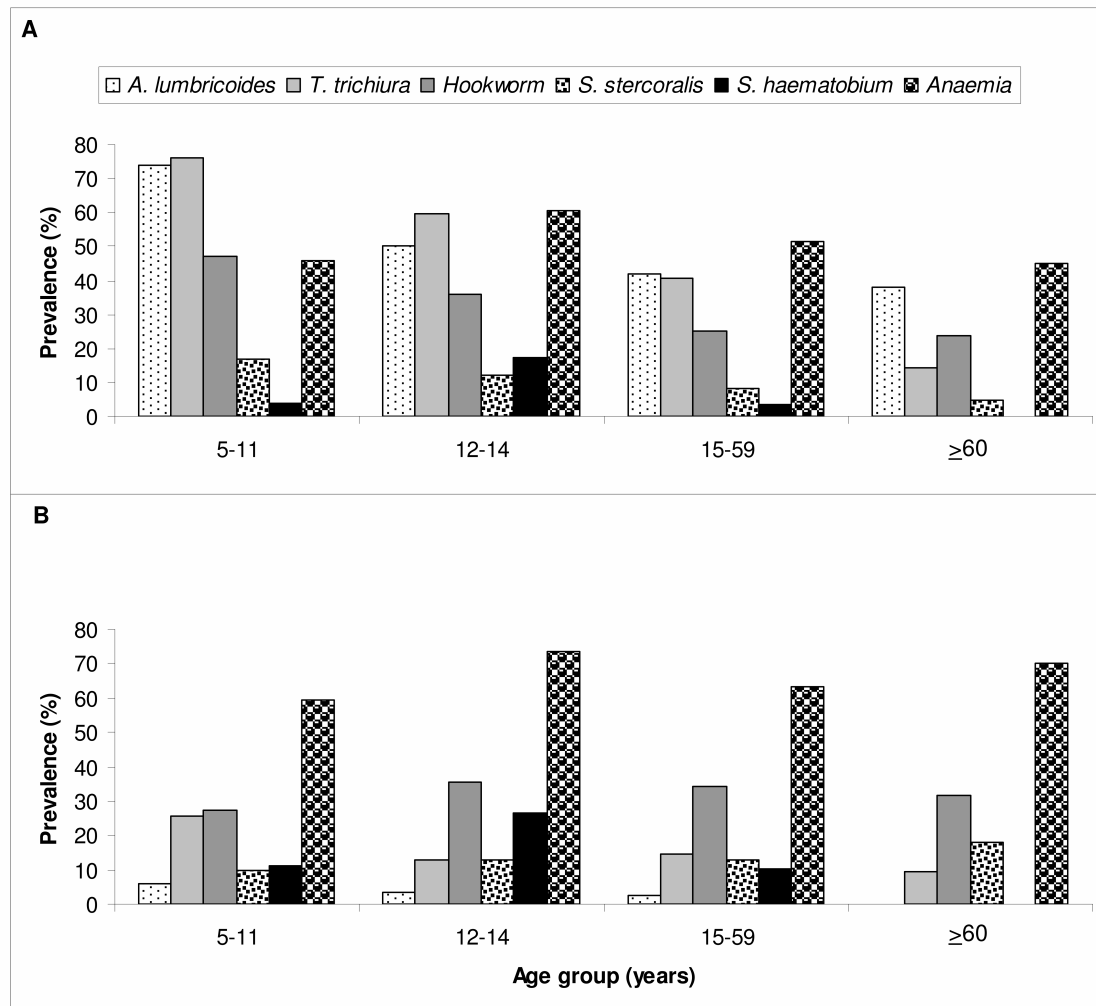


Figure 14. Prevalence of soil-transmitted helminths, *S. haematobium* and anemia in rural Bandamaji and peri-urban Dole, Zanzibar, in June/July 2008. Bar chart indicating the prevalence of soil-transmitted helminths, *S. haematobium* and anemia in four age groups of (A) rural Bandamaji, and (B) peri-urban Dole. (A) age group 5-11 years: n = 55; age group 12-14 years: n = 42; age group 15-59 years: n = 152 ; age group ≥60 years: n = 21. (B) age group 5-11 years: n = 51; age group 12-14 years: n = 32; age group 15-59 years: n = 79; age-group ≥60 years: n = 22.

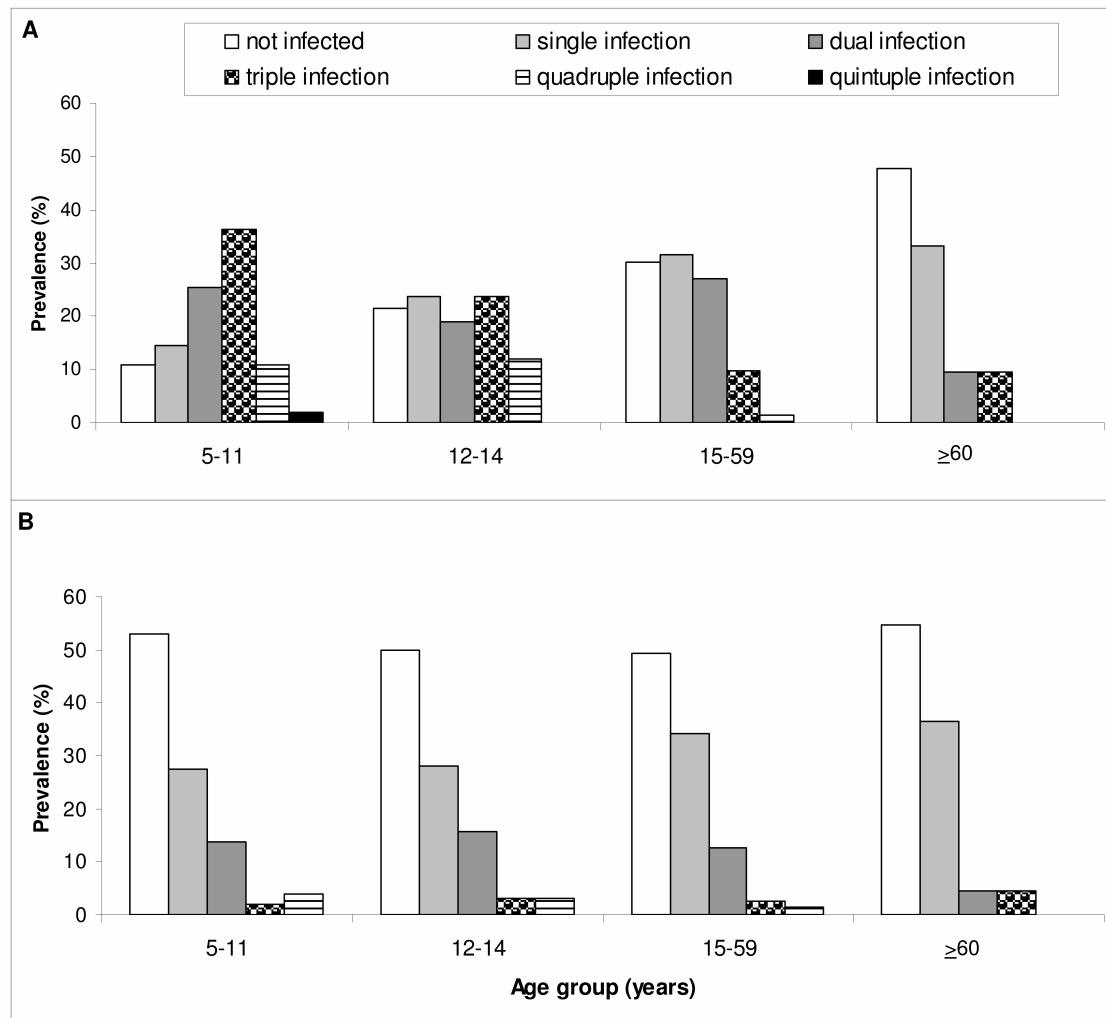


Figure 15. Polyparasitism in rural Bandamaji and peri-urban Dole, Zanzibar, in June/July 2008. Bar chart indicating the number of infecting helminths (polyparasitism of soil-transmitted helminths plus *S. haematobium*) in four age groups of (A) rural Bandamaji and (B) peri-urban Dole. (A) age group 5-11 years: n = 55; age group 12-14 years: n = 42; age group 15-59 years: n = 152 ; age-group ≥60 years: n = 21. (B) age group 5-11 years: n = 51; age group 12-14 years: n = 32; age group 15-59 years: n = 79; age-group ≥60 years: n = 22.

Considering only arithmetic mean EPGs from infected individuals, the box plots in Figure 16 indicate that EPGs for *A. lumbricoides* were significantly higher in 5-11-year-old children than in participants aged 15-59 years (Figure 16A). EPGs for *T. trichiura* were significantly higher in the 5-11-year-old children than in the three older age groups (Figure 16B). In contrast, EPGs for hookworm showed no significant difference between age groups (Figure 16C).

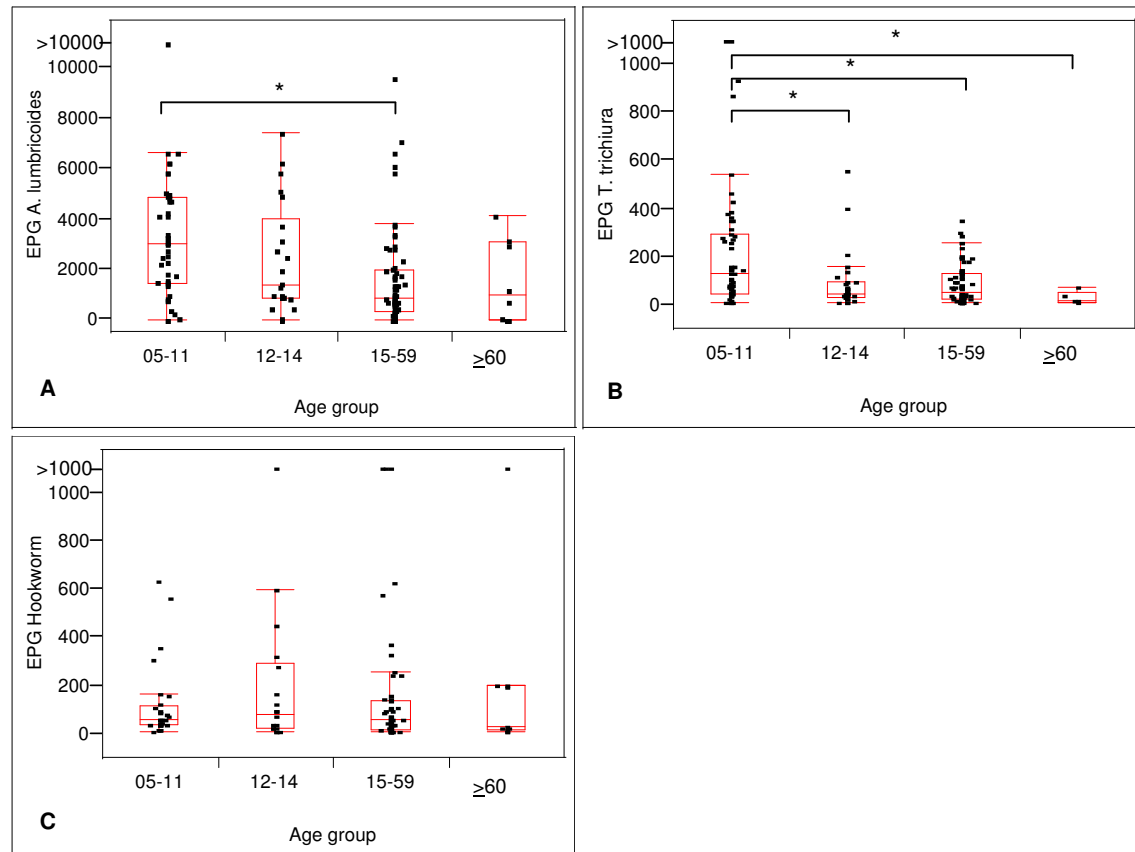


Figure 16. Age group specific differences in helminth infection intensities as expressed by egg excretion. Eggs per gram (EPG) values of infected individuals in different age groups of two Zanzibari communities: rural Bandamaji and peri-urban Dole. Each person's EPG was estimated as the arithmetic mean of at least two Kato-Katz thick smear readings. Differences in the median EPG of the four age groups were determined using the Kruskal-Wallis test. Pair-wise comparisons between the median EPG of two age groups were adjusted for multiple testing as suggested by Siegel and Castellan (1988) [35]. Horizontal bars are indicating the significant differences of the median EPG between two groups. Box plot: the ends of the box represent the 75th and 25th percentiles; the middle line represents the median; the upper whisker represents the upper quartile + 1.5*(interquartile range); the lower whisker represents the lower quartile – 1.5*(interquartile range).

(A): EPG values of *A. lumbricoides* infections, Kruskal-Wallis test: $p < 0.001$; age group 5-11 years: $n = 43$; age group 12-14 years: $n = 22$; age group 15-59 years: $n = 66$; age-group ≥ 60 years: $n = 8$.

(B) EPG values of *T. trichiura* infections, Kruskal-Wallis test: $p < 0.001$; age group 5-11 years: $n = 55$; age group 12-14 years: $n = 29$; age group 15-59 years: $n = 74$; age-group ≥ 60 years: $n = 5$.

(C) EPG values of hookworm infections; Kruskal-Wallis test: $p = 0.789$; age group 5-11 years: $n = 28$; age group 12-14 years: $n = 18$; age group 15-59 years: $n = 53$; age-group ≥ 60 years: $n = 10$.

14.5.5. Seroprevalence of helminth infections

Positive antibody reactions against *A. lumbricoides* antigen were found in all but one tested individual (99.6%). The seroprevalence of anti-*S. haematobium* antibodies was significantly higher in Bandamaji than in Dole (46.1% versus 24.8%; $\chi^2 = 14.1$, $p < 0.001$). The seroprevalence of *S. stercoralis* infections was 32.9% in Bandamaji and 12.8% in Dole, which revealed a highly significant difference ($\chi^2 = 15.3$, $p < 0.001$). With regard to *S. haematobium*, four individuals who were found with eggs in their urine showed negative ELISA test results. Finally, 24 individuals were tested positive for *S. stercoralis* either with the KAP, or the BM, or both methods, but antibody reactions in the ELISA were judged absent. The sensitivities of the *A. lumbricoides*, *S. haematobium*, and *S. stercoralis* ELISAs were 100%, 81.8%, and 38.5%, respectively, and the specificities were 0.6%, 63.9%, and 75.0%, respectively.

14.5.6. Risk factors for helminth infections and anemia, stratified by study setting

Table 16 summarizes the statistically significant ($p < 0.05$) risk factors for helminth infections and anemia determined by multivariable logistic regression modeling, stratified by study setting. In rural Bandamaji, males had an increased risk of an *A. lumbricoides* infection (OR = 1.94, 95% CI 1.03-3.65). An incremental increase of age by 1 year reduced the risk of an *A. lumbricoides* infection (OR = 0.98, 95% CI 0.96-0.99). People consuming raw vegetables or salad were more likely to be infected with *A. lumbricoides* (OR = 2.54, 95% CI 1.27-5.10). In peri-urban Dole, no significant risk factors for an *A. lumbricoides* infection were determined.

Participants from rural Bandamaji belonging to the least poor wealth quintile were at a significantly lower risk of a *T. trichiura* infection than their counterparts belonging to the most poor wealth quintile (OR = 0.28, 95% CI 0.10-0.82). In peri-urban Dole, washing hands after defecation was determined as a protective factor against a *T. trichiura* infection (OR = 0.06, 95% CI 0.01-0.26). In both study settings, for an incremental increase of age by 1 year, the risk of a *T. trichiura* infection decreased (Bandamaji: OR = 0.96, 95% CI 0.94-0.97; Dole: OR = 0.97, 95% CI 0.94-1.00).

Males from Bandamaji had an increased risk of a hookworm infection (OR = 2.25, 95% CI 1.23-4.12). In Dole, people with a recent travel history were more likely to be infected with hookworm (OR = 5.06, 95% CI 1.21-21.06). Belonging to the very poor (OR = 0.11, 95% CI 0.02-0.58) or least poor wealth quintile (OR = 0.12, 95% CI 0.04-0.42) and consumption of

unpeeled fruits (OR = 0.28, 95% CI 0.11-0.73) were protective factors against a hookworm infection in Dole.

In rural Bandamaji, an incremental increase of age by 1 year reduced the risk of a *S. stercoralis* infection (OR = 0.97, 95% CI 0.94-1.00). In peri-urban Dole, males were significantly more likely to be infected with *S. stercoralis* than females (OR = 4.11, 95% CI 1.21-13.90). Moreover, a recent travel history increased the risk of a *S. stercoralis* infection in Dole (OR = 5.43, 95% CI 1.08-27.27), whereas washing hands after defecation was a protective factor (OR = 0.29, 95% CI 0.09-0.96).

In both communities an incremental increase of age by 1 year was associated with a lower risk of a *S. haematobium* infection (Bandamaji: OR = 0.93, 95% CI 0.90-0.95; Dole: OR = 0.97, 95% CI 0.95-1.00). Males from Bandamaji were less likely to be anemic than females (OR = 0.51, 95% CI 0.27-0.94), and consumption of raw vegetables or salad was a protective factor against anemia (OR = 0.45, 95% CI 0.22-0.93). In Dole, no significant risk factors for anemia were found.

Table 16. Risk factors significantly ($p < 0.05$) associated with helminth infections and anemia in individuals from rural Bandamaji and peri-urban Dole in Zanzibar, in June/July 2008, as determined with multivariate logistic regression modeling. The original models included the following explanatory variables wherever expedient: the demographic variables sex and age, wealth quintiles, the risk factors washing hands with soap before eating, washing hands after defecation, washing hands with soap after defecation, consumption of raw vegetables or salad, consumption of unpeeled fruits, consumption of soil (only >5% in Bandamaji), always wearing shoes, recent travel history (only >5% in Dole), having a private toilet, sleeping under a bed net, owning a cat (only >5% in Dole), and owning a dog (only >5% in Dole). Stepwise backwards logistic regression was performed keeping only explanatory variables with P-values <0.2.

Parasite	Community	Risk factor	Adjusted OR ^a	(95% CI) ^b	Wald-test P-value
<i>A. lumbricoides</i>	Bandamaji ^c	Male	1.94	(1.03, 3.65)	0.039
		Age	0.98	(0.96, 0.99)	0.004
		Eating raw vegetables or salad	2.54	(1.27, 5.10)	0.009
<i>T. trichiura</i>	Dole ^d	Age	0.97	(0.94, 1.00)	0.027
		Washing hands after defecation	0.06	(0.01, 0.26)	<0.001
	Bandamaji ^e	Least poor	0.28	(0.10, 0.82)	0.020
		Age	0.96	(0.94, 0.97)	<0.001
Hookworm	Dole ^f	Recent travel history	5.06	(1.21, 21.06)	0.026
		Very poor	0.11	(0.02, 0.58)	0.010
		Least poor	0.12	(0.04, 0.42)	0.001
		Eating unpeeled fruits	0.28	(0.11, 0.73)	0.009
	Bandamaji ^g	Male	2.25	(1.23, 4.12)	0.008
<i>S. stercoralis</i>	Dole	Male	4.11	(1.21, 13.90)	0.023
		Washing hands after defecation	0.29	(0.09, 0.96)	0.042
		Recent travel history	5.43	(1.08, 27.27)	0.040
	Bandamaji ^h	Age	0.97	(0.94, 1.00)	0.039
<i>S. haematobium</i>	Dole	Age	0.97	(0.95, 1.00)	0.022
	Bandamaji	Age	0.93	(0.90, 0.95)	<0.001
Anemia	Bandamaji ⁱ	Male	0.51	(0.27, 0.94)	0.032
		Eating raw vegetables or salad	0.45	(0.22, 0.93)	0.032

^aOR = odds ratio. ^bCI = confidence interval. ^cAdjusted for wealth quintiles 2-5, always wearing shoes, and washing hands with soap after defecation. ^dAdjusted for consumption of raw vegetables or salad, wealth quintiles 2-5, and always wearing shoes. ^eAdjusted for wealth quintiles 2-4. ^fAdjusted for sex, wealth quintiles 3 and 4, and washing hands with soap after defecation. ^gAdjusted for age, consumption of soil, and always wearing shoes. ^hAdjusted for sex. ⁱAdjusted for consumption of soil.

14.5.7. Association between helminth infections and anemia, stratified by study setting

As indicated in Table 17, an *A. lumbricoides* infection showed a significant positive association with a *T. trichiura* infection in both communities (Bandamaji: OR = 6.40, 95% CI 3.40-12.06; Dole: OR = 17.28, 95% CI 2.73-109.19). Conversely, a *T. trichiura* infection was significantly associated with an *A. lumbricoides* infection in both study settings (Bandamaji: OR = 5.38, 95% CI 2.74-10.55; Dole: OR = 20.84, 95% CI 3.92-110.75). In Dole, people with a *T. trichiura* infection were likely to harbor a concurrent *S. stercoralis* infection (OR = 5.34, 95% CI 1.39-20.56). A hookworm infection showed a significant positive association with a *T. trichiura* infection in Bandamaji (OR = 2.95, 95% CI 1.56-5.59) and with a *S. haematobium* infection in Dole (OR = 6.84; 95% CI 1.91-24.49). The multivariable regression models also showed that a *S. stercoralis* infection was positively associated with a *T. trichiura* infection (OR = 4.05, 95% CI 1.23-13.27), and that a *S. haematobium* infection was positively associated with a hookworm infection (OR = 6.89, 95% CI 1.80-26.43) in Dole. In general, heavy *S. haematobium* infections showed a strong positive association with hookworm infections (OR = 13.09; $p = 0.008$), and a negative association with *T. trichiura* infections (OR = 0.08; $p = 0.013$). Participants with an *A. lumbricoides* infection had a decreased risk of anemia in Bandamaji (OR = 0.55, 95% CI 0.31-0.98).

Table 17. Significant associations ($p < 0.05$) between different helminth infections and anemia in residents from rural Bandamaji and peri-urban Dole in Zanzibar, in June/July 2008, as determined with multivariate logistic regression modeling. The original models included the following explanatory variables wherever expedient: the demographic variables sex and age, wealth quintiles, infection with *A. lumbricoides*, *T. trichiura*, hookworm, *S. stercoralis*, and *S. haematobium*, and anemia. Stepwise backwards logistic regression was performed keeping only explanatory variables with P-values < 0.2 .

Parasite	Community	Risk factor	Adjusted OR ^a	(95% CI ^b)	Wald-test P-value
<i>A. lumbricoides</i>	Dole ^c	<i>T. trichiura</i>	17.28	(2.73, 109.19)	0.002
	Bandamaji ^d	<i>T. trichiura</i>	6.40	(3.40, 12.06)	< 0.001
<i>T. trichiura</i>	Dole ^e	<i>A. lumbricoides</i>	20.84	(3.92, 110.75)	< 0.001
		<i>S. stercoralis</i>	5.34	(1.39, 20.56)	0.015
	Bandamaji ^f	<i>A. lumbricoides</i>	5.38	(2.74, 10.55)	< 0.001
Hookworm	Dole ^g	<i>S. haematobium</i>	6.84	(1.91, 24.49)	0.003
	Bandamaji ^h	<i>T. trichiura</i>	2.95	(1.56, 5.59)	0.001
<i>S. stercoralis</i>	Dole ⁱ	<i>T. trichiura</i>	4.05	(1.23, 13.27)	0.021
<i>S. haematobium</i>	Dole ^j	Hookworm	6.89	(1.80, 26.43)	0.005
Anemia	Bandamaji ^k	<i>A. lumbricoides</i>	0.55	(0.31, 0.98)	0.043

^aOR = odds ratio. ^bCI = confidence interval. ^cAdjusted for sex. ^dAdjusted for hookworm infection. ^eAdjusted for sex, age, and wealth quintile 5. ^fAdjusted for age, wealth quintile 2, and hookworm infection. ^gAdjusted for age, wealth quintiles 2-5, and *A. lumbricoides* infection. ^hAdjusted for sex and *S. haematobium* infection. ⁱAdjusted for sex. ^jAdjusted for age and *T. trichiura* infection. ^kAdjusted for sex and hookworm infection.

14.5.8. Association between helminth infections or anemia and self-reported morbidity signs

Adjusting for demographic variables with a P-value of 0.2 or lower and stratification by community, we observed the following associations between helminth infections or anemia with self-reported morbidity signs (recall period: 2 weeks): participants from Dole with an *A. lumbricoides* (OR = 22.75, 95% CI 2.50-206.99) or *S. stercoralis* (OR = 4.47, 95% CI 1.01-19.69) infection had an increased risk of an itching body (Table 18). Participants from Bandamaji with an *A. lumbricoides* infection had a decreased risk of coughing (OR = 0.53, 95% CI 0.30-0.95), and those infected with *T. trichiura* had a decreased risk of vomiting (OR = 0.24, 95% CI 0.06-0.96). In Dole, a *T. trichiura* infection increased the risk of stomach ache (OR = 3.31, 95% CI 1.05-10.43). Participants from Bandamaji with anemia had an increased risk of an itching body (OR = 5.35, 95% CI 1.65-17.36) and an increased risk of reporting malaria (OR = 4.98, 95% CI 1.39-17.84) compared with participants without anemia. In Dole, anemia was a risk factor for fatigue (OR = 2.81, 95% CI 1.14-6.89).

Table 18. Self-reported morbidity signs significantly ($p < 0.05$) associated with helminth infections and anemia among residents from rural Bandamaji and peri-urban Dole in Zanzibar, in June/July 2008, as determined with multivariate logistic regression modeling. The original models included the following explanatory variables: the demographic variables sex and age, and wealth quintiles, infection with *A. lumbricoides*, *T. trichiura*, hookworm, *S. stercoralis*, *S. haematobium*, and anemia. Stepwise backwards logistic regression was performed keeping only explanatory variables with P-values < 0.2 .

Reported morbidity sign	Community	Risk factor	Adjusted OR ^a	(95% CI ^b)	Wald-test P-value
Fatigue	Dole ^c	Anemia	2.81	(1.14, 6.89)	0.024
Stomach ache	Dole ^d	<i>T. trichiura</i>	3.31	(1.05, 10.43)	0.041
Vomiting	Bandamaji ^e	<i>T. trichiura</i>	0.24	(0.06, 0.96)	0.044
Cough	Bandamaji ^f	<i>A. lumbricoides</i>	0.53	(0.30, 0.95)	0.033
Itching	Dole ^g	<i>S. stercoralis</i>	4.47	(1.01, 19.69)	0.048
		<i>A. lumbricoides</i>	22.75	(2.50, 206.99)	0.006
	Bandamaji ^h	Anemia	5.35	(1.65, 17.36)	0.005
Malaria	Bandamaji ⁱ	Anemia	4.98	(1.39, 17.84)	0.014

^aOR = odds ratio. ^bCI = confidence interval. ^cAdjusted for age. ^dAdjusted for sex, anemia, and *S. stercoralis*.

^eAdjusted for age. ^fAdjusted anemia. ^gAdjusted for age, anemia, *T. trichiura*, and hookworm infection. ^hAdjusted for age. ⁱAdjusted for age.

14.6. Discussion

Control programs for soil-transmitted helminthiasis, schistosomiasis, and lymphatic filariasis have been implemented in Zanzibar for several years (Mohammed et al., 2008; Knopp et al., 2009). The key strategy is chemotherapy-based morbidity control, using albendazole or mebendazole against soil-transmitted helminthiasis, praziquantel against schistosomiasis, and ivermectin plus albendazole against lymphatic filariasis. Importantly, the drugs used in the GPELF also show an effect against strongyloidiasis (i.e., ivermectin (Marti et al., 1996)) and against soil-transmitted helminthiasis (i.e., albendazole (Keiser and Utzinger, 2008)). The helminth control programs in Zanzibar are considered successful public health interventions because of significant reductions in the prevalence and intensity of helminth infections and high levels of treatment coverage (Mohammed et al., 2006; Knopp et al., 2009; Stothard et al., 2009a).

Analysis of our data showed, however that infections with soil-transmitted helminths and *S. haematobium* are still common, particularly in the rural setting of Bandamaji, where almost three-quarter of the participants were infected with at least one helminth species. Multiple

species helminth infections affected almost half of the participants in Bandamaji, but only about one out of six individuals in the peri-urban setting of Dole. Importantly though, infection intensities were mainly low with highest EPGs observed in the youngest age group (children aged 5-11 years). Seroprevalences according to ELISA tests were 99.6% for *A. lumbricoides*, 39.2% for *S. haematobium*, and 26.4% for *S. stercoralis*, but the test specificities were low. More than half of the participants were anemic and, interestingly, the overall prevalence of anemia in peri-urban Dole was significantly higher than in rural Bandamaji (64.7% versus 50.9%).

Two important limitations of our study are that we did not adhere to a strict randomization procedure for enrollment, and that the number of fully complying individuals was rather low. According to estimates for the year 2007, the total population in Bandamaji and Dole were 1,118 and 2,876, respectively. Hence, our final study cohort consisted of approximately 30% of the population of Bandamaji and 13% in Dole. With regard to the number of fully complying individuals, one should bear in mind that we aimed at collecting three consecutive stool samples per person, employing a suite of diagnostic methods, and that we worked with all age groups of two communities. Repeated stool sampling reduced the study compliance from 79% to 69% for the submission of the first to the second stool sample, and to a level of 48% for submission of all three stool samples. The overall compliance rate is different to the one we reached with repeated stool sampling among school children in two schools in Zanzibar (85%) (Knopp et al., 2008a). School children are, however, readily accessible and the education system provides a convenient platform for deworming campaigns, whereas in the community, research teams and program officers depend on the will and stamina of the individuals not to forget submitting a filled stool container every morning without a daily reminder. The low compliance to the questionnaire survey and concomitant provision of a blood and urine sample is likely a result of the time consuming procedure, competing with other daily activities.

Since treatment coverage by the GPELF implemented from 2001-2006 in both study sites was equally high (mean in Bandamaji: 81.9%, mean in Dole: 83.0%) and school-based deworming reached coverage levels of 75% of the at-risk population in Zanzibar in 2006, there must be other local risk factors abetting different levels of helminth infections and anemia. The distinctive age-dependent patterns of *A. lumbricoides*, *T. trichiura*, and *S. haematobium* infection prevalence and intensity in our study population are consistent with the literature (Hotez et al., 2006a). All other identified risk factors in our study showed setting-specific idiosyncrasies. For example, *S. stercoralis* infections showed no clear age-

profile, but males were at a several-fold higher risk of an infection than females in Dole, similar to the observed gender difference for hookworm infection in Bandamaji. It should be noted that reports on the prevalence and age-profile of *S. stercoralis* infections are rare, often with conflicting results. Whilst studies from Côte d'Ivoire and China reported a higher prevalence of strongyloidiasis in adults (Dancesco et al., 2005; Steinmann et al., 2007), in the Peruvian Amazon and in aboriginal communities in Australia mostly children were affected (Prociv and Luke, 1993; Egidio et al., 2001). In line with findings from Jamaica, our data suggest that an infection with *S. stercoralis* is independent of age (Lindo et al., 1995). The observation of a higher *S. stercoralis* prevalence among males confirms results obtained elsewhere (Arakaki et al., 1992; Egidio et al., 2001), but is in contrast to recent findings from Côte d'Ivoire (Glinz et al., 2009). The higher risk of both hookworm and *S. stercoralis* infections in males is likely a result of genetic and immunologic determinants, as well as of gender-specific risk behavior. Moreover, and depending on the study setting, several behavioral factors were identified that showed significant associations with helminth infections and anemia: consumption of raw vegetable or salad was a risk factor for an infection with *A. lumbricoides*, whereas washing hands after defecation and socio-economic status (least poor wealth quintile) were significant protective factors against a *T. trichiura* infection. People washing hands after defecation were also less likely to be infected with *S. stercoralis*. A recent travel history was associated with a higher risk of both hookworm and *S. stercoralis* infection. Males and the participants consuming raw vegetables or salad were at a lower risk of anemia. In addition to age, sex, socio-economic status, and personal behavior, we believe that there are setting-specific sanitary and environmental risk factors that might abet helminth infections. For example, in District North A, 46% of all households had no toilet facilities in 2004/2005, whereas in District West only 8% of households had no access to toilet facilities (MoHSW, 2007). Hence, the environmental contamination with helminth eggs or larvae is likely to be higher in District North A, and hence people are at a higher risk of soil-transmitted helminth infections. Moreover, the survival and longevity of helminth eggs or larvae in the natural environment depend on soil type and vegetation, which has previously been discussed for Zanzibar and other African settings (Saathoff et al., 2005a; Saathoff et al., 2005b; Knopp et al., 2008b).

The positive associations between (i) *A. lumbricoides* and *T. trichiura*, (ii) hookworm and *T. trichiura*, (iii) *S. stercoralis* and *T. trichiura*, and (iv) hookworm and *S. haematobium* observed in the current study in Unguja are in line with previous investigations on helminth associations carried out in Unguja and Pemba (Booth et al., 1998; Stothard et al., 2009a).

Interestingly, the adjusted OR indicating an association of hookworm and *S. haematobium* infection increased from 4.3 to 13.1 if children harbored heavy *S. haematobium* infections. This observation is similar to findings from Côte d'Ivoire and Brazil where children with increasing infection intensity of *S. mansoni* were also more likely to be concurrently infected with hookworm (Chamone et al., 1990; Keiser et al., 2002). Hookworm and *Schistosoma* spp. infections are leading causes of anemia that can result in growth retardation and cognitive impairment of children (Friedman et al., 2005; King et al., 2005; Hotez et al., 2008). Hence, if hookworm infections are associated with or even exacerbate with a concurrent schistosome infection, the risk of chronic anemia and related morbidity is likely to be elevated in co-endemic settings. To control morbidity due to multiple helminth infections, triple co-administration of albendazole, praziquantel, and ivermectin might be an effective strategy. Triple co-administration of the respective drugs has been shown to be safe in co-endemic settings in Zanzibar, where multiple rounds of treatment had been implemented in the past (Mohammed et al., 2008). Since demographic factors, personal risk behavior, and socio-economic status shape the profile of helminth infections, poverty alleviation strategies complemented with health education and improved access to clean water and adequate sanitation, in addition to regular deworming, can help to decrease the burden of helminth infections in Africa and elsewhere in the developing world (Hotez et al., 2006b; Utzinger et al., 2009).

The high prevalence rates of anemia observed in both settings of our study indicate that anemia is still a major public health problem in Zanzibar, which begins early in life (Sousa-Figueiredo et al., 2008). Participants from the rural setting presenting with anemia were five times more likely to report a malaria infection within the last two weeks. The association between malaria and anemia is well documented (Menendez et al., 2000). Interestingly, people infected with one or several helminth species concurrently were not at a higher risk of anemia compared with non-infected individuals. Our results are therefore in contrast to a study carried out in the Philippines, where individuals with multiple species helminth infections of light intensity were at an elevated risk of anemia (Ezeamama et al., 2005). In the current study, participants from Bandamaji infected with *A. lumbricoides* were at a lower risk of anemia and cough, and those infected with *T. trichiura* were less likely to report vomiting within the past two weeks. These findings might point to a potent immuno-modulation by helminths resulting in disease protective immune responses (Maizels et al., 2004). Since all findings were setting-specific it is, however, also conceivable that the apparent associations were due to local social or environmental determinants, as suggested elsewhere (Mwangi et

al., 2006). We were surprised to find a higher prevalence of anemia in the peri-urban community than in rural Bandamaji. Our results might suggest that anemia was driven not only by malaria but also by nutritional factors, and perhaps ethnicity, rather than by (multi)helminth infections (Zimmermann and Hurrell, 2007; Pradhan, 2009).

Sero-prevalences of *A. lumbricoides*, *S. stercoralis*, and *S. haematobium*, as determined by ELISA, were several-fold higher than the prevalences found with standard direct diagnostic methods for eggs and larvae, but it must be emphasized that the specificities of all performed ELISAs were low. Likely reasons for these observations are as follows. First, whilst the prevalences and intensity of helminth infections have significantly decreased as a result of large-scale deworming programs in Zanzibar (Knopp et al., 2009), antibodies from past infections can persist for a prolonged period of time after successful treatment, and hence be detected with ELISA (Bhattacharyya et al., 2001; Duus et al., 2009). Second, it is known that unspecific cross-reactions (e.g., from antibodies against antigen from *Ascaris* or filarial worms) can occur (Neppert, 1974; Chatterjee et al., 1996). Third, widely used parasitological techniques such as the K-K thick smear lack sensitivity for detecting low-intensity helminth infections (Booth et al., 2003; Knopp et al., 2008a). Indirect diagnostic tools such as ELISA might be more sensitive (Doenhoff et al., 2004; van Doorn et al., 2007; Stothard et al., 2009b).

While there are many explanations for higher helminth seroprevalences determined with ELISA, it is unclear why the ELISAs failed to detect four *S. haematobium* and 24 *S. stercoralis* infections diagnosed by microscopy. Most study participants, however, had either a very low antibody response to all performed ELISAs or the measured OD was marginal below the cut-off level, suggesting that the thresholds proposed in the manufacturer's manual need careful revision, at least for our study setting. Summarizing, our data confirm that people living in areas highly endemic for helminthiases are immunologically activated as a result of previous infections (Fincham et al., 2007). Since helminths are masters in modulating host immunity they are likely impacting on co-infections, allergy, and immunizations (Maizels et al., 2004). Therefore, it will be important to incorporate gained knowledge on the epidemiology of immunological markers in future public health decisions.

Our study indicates that, despite considerable progress made in the control of helminthiases in Zanzibar (Mohammed et al., 2006; Knopp et al., 2009; Stothard et al., 2009a), the “worm-problem” and anemia in Zanzibar remains a formidable challenge and cannot be overcome by preventive chemotherapy alone (WHO, 2006b). It should be noted that the GPELF, which regularly deployed albendazole plus ivermectin, likely had a beneficial

effect on soil-transmitted helminthiasis, was terminated in 2006. The patterns of helminth infections and anemia in rural and peri-urban communities and the identified risk factors emphasize that the pressure of helminth transmission in Zanzibar is still pervasive and that additional control measures are needed to consolidate progress made to date with preventive chemotherapy. With new discussions exploring options for further reduction of helminthiasis in Zanzibar and elsewhere, including shifting the focus from morbidity control to transmission control, there is a need for integrated control programs, acting beyond preventive chemotherapy (Annonymus, 2004; Utzinger and de Savigny, 2006; Utzinger et al., 2009). Indeed, greater steps should be taken to enforce health education and action is needed to improve access to clean water and adequate sanitation (e.g., community-led total sanitation). These measures will also result in an enhanced socio-economic status of people, and hence alleviate poverty, which is the key factor for the control and ultimate elimination of helminthiasis.

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14.9. Translation of abstract into German

Muster und Risikofaktoren von Wurminfektionen in einer ländlichen und einer peri-urbanen Bevölkerung in Sansibar, im Kontext von Wurmkontrollprogrammen

Hintergrund: Die Kontrolle von Wurminfektionen und die Vorbeugung von Blutarmut sind wichtig zur Verbesserung des Gesundheitswesens in Entwicklungsländern. Es war unser Ziel die Muster und Risikofaktoren von Wurminfektionen und Blutarmut in einer ländlichen und in einer peri-urbanen Gemeinde in Sansibar, Tansania, im Kontext von nationalen Wurmkontrollprogrammen zu beschreiben.

Methoden/wichtigste Ergebnisse: Wir führten eine Querschnittsstudie mit 454 Personen aus zwei Dorfschaften durch und untersuchten mindestens zwei Stuhlproben mit unterschiedlichen Methoden auf parasitäre Würmer (Rundwurm, Hakenwurm, Zwergfadenwurm, und Peitschenwurm) und eine Urinprobe auf den Erreger der Blasenbilharziose (*Schistosoma haematobium*). Kapillares Blut aus dem Finger wurde benutzt um Blutarmut und Antikörperreaktionen gegen Rundwurm, Zwergfadenwurm und Blasenbilharziose mit immunologischen Tests festzustellen. Bei rund drei Vierteln der ländlichen Dorfschaft (73.7%) und bei knapp der Hälfte der peri-urbanen Bevölkerung (48.9%) diagnostizierten wir Wurminfektionen. Die meisten Infektionen waren leicht und Kinder im Schulalter waren am stärksten infiziert. Infektionen mit mehreren Wurmart gleichzeitig waren unabhängig vom Alter in der ländlichen Bevölkerung häufiger. Mehr als die Hälfte der Studienteilnehmer und besonders die peri-urbane Bevölkerung litt unter Blutarmut. Risikofaktoren für Wurminfektionen waren Alter, Geschlecht, der Verzehr von rohem Gemüse oder Salat, Reisen ausserhalb der Gemeinde, und der sozioökonomische Status.

Schlussfolgerung/Bedeutung: Seit mehreren Jahren verfolgt Sansibar ein nationales Wurmkontrollprogramm, welches vorwiegend auf medikamentöser Behandlung zur Vorbeugung von Erkrankungen beruht. Wurmerkrankungen und Blutarmut sind allerdings immer noch häufig. Die Intensitäten der Infektionen sind hingegen niedrig. Aus diesem Grund schlagen wir vor, dass die Behandlung fortgeführt wird. Um eine nachhaltigere Wirkung zu erzielen sollten aber im Einklang mit weiteren Armutsbekämpfungsmaßnahmen auch der Zugang zu sauberem Trinkwasser, adäquaten sanitären Einrichtungen und Gesundheitserziehung verbessert werden.

15. Changing patterns of soil-transmitted helminthiasis in Zanzibar in the context of national helminth control programs

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15.1. Abstract

Helminth control programs have been implemented in Zanzibar for over a decade. In June/July 2007, approximately six months after the last anthelmintic treatment, a cross-sectional survey was carried out in two schools and results were compared with data obtained in the same schools in 1994. Multiple stool samples collected from 368 school children were subjected to the Kato-Katz, Koga agar plate and Baermann methods. The prevalence of *Trichuris trichiura*, hookworm, *Ascaris lumbricoides* and *Strongyloides stercoralis* was 46.6%, 21.6%, 16.9% and 10.2%, respectively. Infection intensities were generally low. Compared to 1994, the prevalence of *S. stercoralis*, hookworm, *A. lumbricoides* and *T. trichiura* decreased by 81.0%, 80.5%, 70.6% and 48.6%, respectively. Infection intensities declined by > 95% for all helminth species investigated. Our study confirms that preventive chemotherapy successfully reduces the level and intensity of helminth infections. To consolidate achievements made, additional control measures such as health education and environmental sanitation are needed.

Keywords: Soil-transmitted helminthiasis, *Ascaris lumbricoides*, hookworm, *Strongyloides stercoralis*, *Trichuris trichiura*, treatment, control, Zanzibar, Tanzania

15.2. Introduction

Infections with soil-transmitted helminths are common across sub-Saharan Africa and elsewhere in developing nations (Brooker et al., 2006; Hotez et al., 2008). Soil-transmitted helminth infections typically afflict the poorest population segments and impact on human health, nutrition and worker productivity, and hence exacerbate poverty (Savioli et al., 2004). Efforts are under way to control diseases caused by chronic infections with soil-transmitted helminths. The most widely used strategy is morbidity control by means of preventive chemotherapy, which is the large-scale application of anthelmintic drugs (e.g., albendazole and mebendazole), usually to school-aged children without prior diagnosis (Brooker et al., 2006; Hotez et al., 2008; Keiser and Utzinger, 2008). Emphasis on school-aged children is justified because high levels of soil-transmitted helminth infections are observed in this age group, and because schools offer a convenient platform to reach those in need for treatment (Worldbank, 2003; Lancet-Editorial, 2004).

In Unguja and Pemba, the two main islands forming Zanzibar, soil-transmitted helminth infections were recognized as a major public health problem in the early 1990s. Indeed, school-aged children were virtually all infected with at least one of the three common soil-transmitted helminths, namely *Ascaris lumbricoides*, *Trichuris trichiura* and hookworm (*Ancylostoma duodenale* and *Necator americanus*) (Marti et al., 1996; Albonico et al., 1997a). Additionally, infections with *Strongyloides stercoralis*, the most neglected soil-transmitted helminth species (Olsen et al., 2009), were found in a third of the children examined in the schools of Chaani and Kinyasini in Unguja (Marti et al., 1996). In 1994, the Ministry of Health and Social Welfare (MoHSW) of Zanzibar, in collaboration with the World Health Organization (WHO), established an action plan for the control of soil-transmitted helminths and urinary schistosomiasis (Montresor et al., 2001). Over the past decade, albendazole, mebendazole and praziquantel have been administered to children in primary schools on a fairly regular basis (Figure 17) (Renganathan et al., 1995; Stothard et al., 2008). For example, in Chaani and Kinyasini, children received annual treatment with mebendazole and praziquantel from 1995 to 2000 via the national helminth control program and, after a shortage of drug donations, albendazole and praziquantel were again distributed from 2003 onwards to school children as part of the “Kick out Kichocho Program” (Mohammed et al., 2008; Stothard et al., 2006; 2009a). Starting in 2001, the Global Program to Eliminate Lymphatic Filariasis (GPELF) targeted eligible individuals in Zanzibar (including children aged 5 years and above) annually with ivermectin plus albendazole (WHO, 2001; Mohammed

et al., 2006; 2008). Of note, ivermectin (single oral dose of 200 µg/kg) is not only efficacious against filarial worms but also against *S. stercoralis* and *A. lumbricoides* (Marti et al., 1996; Zaha et al., 2000). By 2006, Zanzibar and Burkina Faso were the first territories in the WHO African Region achieving the target of regular anthelmintic drug administration to at least 75% of all school-aged children at risk of morbidity (WHO, 2008).

The aim of the present study was to determine the prevalence and intensity of soil-transmitted helminth infections, including *S. stercoralis*, among a random sample of school children in Chaani and Kinyasini, using a rigorous diagnostic approach. The findings were compared to data from 1994 obtained in the same schools in order to study the dynamics of soil-transmitted helminth infections in the face of preventive chemotherapy. The findings from Zanzibar might be of interest to public health specialists in defining and refining end-point targets of present de-worming initiatives.

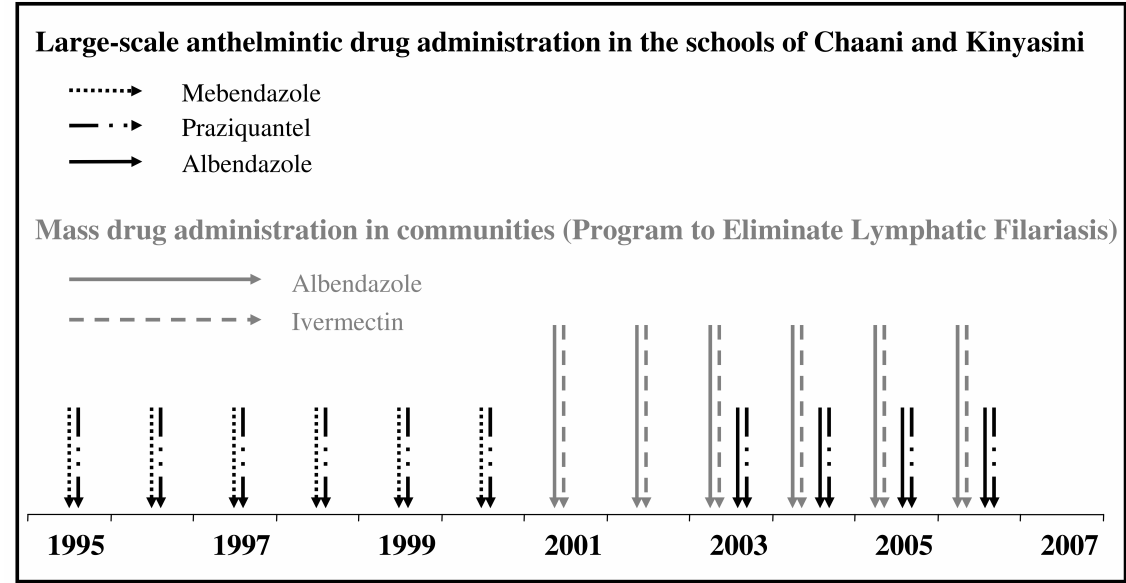


Figure 17. Diagram detailing the treatment with mebendazole (dotted arrowed line), albendazole (arrowed line), ivermectin (dashed arrowed line) and praziquantel (dotted and dashed arrowed line) since the onset of large-scale anthelmintic drug administration in 1995 until the cross-sectional survey reported here in June 2007. The school-based national helminth control program started with mebendazole (500 mg, single oral dose) and praziquantel (40 mg/kg, single oral dose) in 1995 and, after a break due to drug shortage, changed to albendazole (400 mg, single oral dose) and praziquantel (40 mg/kg) in 2003 as part of the “Kick out Kichocho Program”. The program to eliminate lymphatic filariasis started in 2001 distributing ivermectin (200 µg/kg, single oral dose) plus albendazole (400 mg, single oral dose) to the whole eligible population.

15.3. Materials and methods

15.3.1. Study area and population

The study was carried out in June/July 2007 in the schools of Chaani and Kinyasini in Unguja, the main island of Zanzibar, in collaboration with the Helminth Control Laboratory Unguja (HCLU) of the MoHSW. These two schools were selected because a similar survey had been conducted there in 1994, before the launch of national helminth control programs (Marti et al., 1996). Chaani (geographic coordinates: 5° 55' 48" S latitude, 39° 17' 58" E longitude) and Kinyasini (5° 58' 13" S, 39° 18' 30" E) are located 40 km and 35 km northeast from Zanzibar Town. Pupils from both schools were subjected to large-scale administration of anthelmintic drugs, most recently in December 2006. The sample size was calculated using an equation given by Fleiss (Fleiss, 1981). We assumed that the smallest change would have occurred in the prevalence of *S. stercoralis* infections because the benzimidazoles (albendazole and mebendazole) only show a low efficacy in clearing this parasite (Marti et al., 1996; Zaha et al., 2000). With a given prevalence of 30% for *S. stercoralis* infections in 1994 and an estimated prevalence of 20% in 2007, and using an alpha error of 5% to detect a significant difference in prevalences and a power of 80%, the required number of individuals was calculated to be 312. We assumed a return rate (compliance) for providing stool samples of 90% per collection day. In view of the required number of participants, and allocating for participants lost due to collection of multiple stool samples, approximately 400 subjects had to be included in the study.

15.3.2. Field and laboratory procedures

The purpose and procedures of the study were explained in detail to the headmasters and teachers of the schools. Subsequently, a teacher and a member of the HCLU team explained the study to the pupils in lay terms. Based on our sample size calculation, we randomly selected 401 school children from all 7 grades and invited them to provide 3 stool samples over consecutive days. Stool samples were collected in the morning (between 08:00 AM and 09:00 AM), transferred to HCLU and, within 3 hours, processed with the Kato-Katz technique (Katz et al., 1972), the Koga agar plate method (Koga et al., 1991), and the Baermann technique (García and Bruckner, 2001). Detailed descriptions of the methods used have been presented elsewhere (Knopp et al., 2008). In brief, Kato-Katz thick smears (using 41.7 mg templates) were quantitatively examined for *A. lumbricoides*, hookworm and *T. trichiura*

eggs. The number of *S. stercoralis* larvae was quantified with the Baermann technique. Additionally, the presence of *S. stercoralis* and/or hookworm larvae was assessed qualitatively with the Koga agar plate method. A random sample of 5% of the Kato-Katz thick smears was re-examined by a senior technician for quality control.

Marti and colleagues, in 1994, collected a single stool sample from 1204 school children (median age = 14 years) from the schools in Chaani and Kinyasini. Stool samples were subjected to a single Kato-Katz thick smear (41.7 mg template) for quantitative diagnosis of *A. lumbricoides*, hookworm and *T. trichiura*, and a single Baermann test for detection of *S. stercoralis* larvae (Marti et al., 1996).

15.3.3. Statistical analysis

Data were double entered in Excel version 10.0 (edition 2002, Microsoft Corporation) and cross-checked in EpiData version 3.1 (EpiData Association; Odense, Denmark).

For analyses the statistical packages JMP version 5.0.1 (SAS Institute; Cary, NC) and STATA version 9.2 (StataCorp.; College Station, TX) were utilized. Only individuals who submitted ≥ 2 stool samples of sufficient quantity were eligible for subsequent analyses. The helminth species-specific ‘true’ prevalences as well as the sensitivity (i.e., proportion of true positives identified as positive) of the individual diagnostic methods were calculated, using a mathematical model (Marti and Koella, 1993). This model calculates the ‘true’ prevalence by relating the number of stool samples found to be positive for a given helminth species to the number of false-negative results obtained for the same participant upon multiple sampling. To predict the sensitivity of the diagnostic test, the model employs the frequency of positive test results among stool samples submitted by the same individual. The procedure follows an approach developed by Mullen and Prost (Mullen and Prost, 1983), and has been previously employed for estimating the ‘true’ prevalence of soil-transmitted helminths, including *S. stercoralis* (Bogoch et al., 2006; Steinmann et al., 2007; Knopp et al., 2008).

Helminth species-specific egg counts from the Kato-Katz thick smear readings were multiplied by a factor 24 to derive infection intensities, expressed as eggs per gram of stool (EPG). For each individual, the arithmetic mean EPG for each helminth was calculated from the Kato-Katz thick smears. Infection intensities were stratified into light, moderate and heavy, according to thresholds issued by WHO (Montresor et al., 1998; WHO, 2002). For the study cohort, the geometric mean EPG for each helminth species and, for *S. stercoralis*, the

geometric mean larval count was calculated using the formula provided by Montresor and colleagues (Montresor et al., 1998).

A linear regression analysis was used to investigate for an association between EPG and school grade. Pearson's χ^2 test was used to explore for associations between infection and age, sex and school. In univariate and multivariate analyses, odds ratios (ORs) including 95% confidence intervals (CIs), were calculated for age, sex and school. Cases were defined as presence of infection (≥ 1 helminth egg detected in a Kato-Katz thick smear; ≥ 1 larvae detected in the Koga agar plate or Baermann), in at least 1 stool sample. Differences were considered significant at a level of 5%.

15.3.4. Ethical considerations and treatment

Clearance for the study was given by the institutional research commission of the Swiss Tropical Institute (Basel, Switzerland) and the institutional review board of the National Health Service Local Research Ethics Committee (application 03.36) of St. Mary's Hospital (London, UK) on behalf of the Natural History Museum/Imperial College London. The study protocol was approved by WHO, MoHSW and the Ministry of Education of Zanzibar (Zanzibar, Tanzania).

The study was embedded in one of the parasitologic surveys carried out by HCLU in the schools of Unguja. The headmasters and teachers of Chaani and Kinyasini schools were informed about the purpose and procedures of the study. Detailed explanations were given to the school children by trained staff of the HCLU and the teachers. Participation was voluntary and each child could withdraw from the study anytime without further obligation. Parents or legal guardians signed a written informed consent sheet for all anticipated medical interventions, including parasitologic surveys at schools when they registered their children for school attendance. Oral consent to participate in the current study was obtained from all children in the presence of local health and education authorities.

Both schools were subjected to anthelmintic drug administration conducted by HCLU in the following months, where all school children were treated with a single oral dose of albendazole (400 mg) and praziquantel (40 mg/kg) regardless of their infection status. Additionally, all participants diagnosed with a *S. stercoralis* infection were treated with a single oral dose of ivermectin (200 μ g/kg).

15.4. Results

15.4.1. Study profile and compliance

Figure 18 shows the study profile and compliance to submit multiple stool samples. From the 401 randomly selected school children (202 in Kinyasini and 199 in Chaani), 25 did not participate further and 8 submitted only a single stool sample. At least 2 stool samples were obtained from the remaining 368 children (96.3%). Due to insufficient quantities of feces and the priority for the sequence of tests employed, ≥ 2 Kato-Katz thick smear results were available from 367 children, ≥ 2 Koga agar plate readings were done for 366 children, and 364 children had ≥ 2 Baermann results. Complete data records were available for 362 children, resulting in an overall compliance of 90.3%. This final cohort included 209 girls and 153 boys with a median age of 12 years (range: 7-20 years).

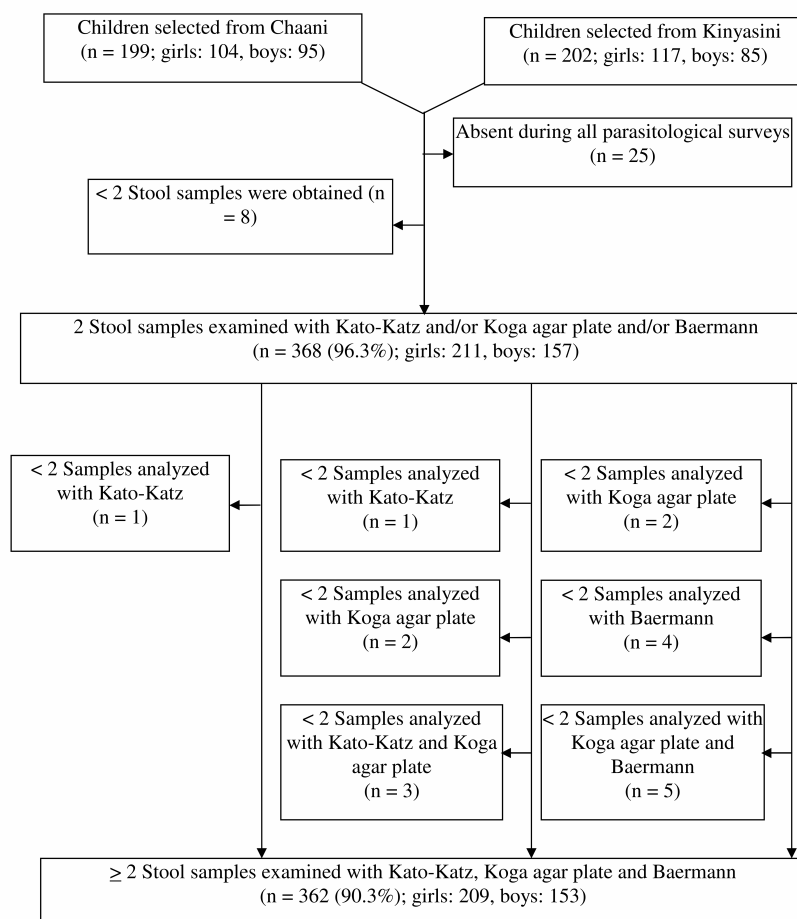


Figure 18. Diagram detailing the study participation and compliance of randomly selected school children from Kinyasini and Chaani, Unguja, Zanzibar. All children providing at least 2 stool samples were included in the final analysis. The final cohort comprised children with complete datasets (i.e., ≥ 2 stool samples examined with 3 methods each).

15.4.2. Prevalence and intensity of helminth infections

Table 19 summarizes the prevalence of helminth infections in relation to the diagnostic method employed and the sampling effort. The prevalence of *T. trichiura* and *A. lumbricoides*, based on ≥ 2 Kato-Katz thick smear readings per individual, was 46.6% and 16.9%, respectively. An overall hookworm prevalence of 21.6% was found, as assessed by the combined results from the Kato-Katz and Koga agar plate method. The prevalence of *S. stercoralis* based on ≥ 2 stool samples subjected to the Baermann and Koga agar plate method was 10.2%. Employing a mathematical model (Marti and Koella, 1993), the ‘true’ prevalence of *T. trichiura*, hookworm, *A. lumbricoides* and *S. stercoralis* was 48.4%, 23.1%, 17.1% and 14.4%, respectively.

The sensitivity for detecting a *S. stercoralis* infection increased by 54.4% when 2 rather than a single stool sample were subjected to the Koga agar plate plus Baermann methods. For hookworm diagnosis, duplicate Kato-Katz thick smears plus duplicate Koga agar plate tests improved the sensitivity by 25.5%, compared to a single stool sample subjected to these methods. The sensitivity of *T. trichiura* and *A. lumbricoides* diagnosis improved by 19.2% and 10.8%, respectively, when 2 Kato-Katz thick smears, rather than a single one, were examined.

Table 20 summarizes the EPGs and *S. stercoralis* larval counts of our final study cohort and indicates that, according to WHO thresholds, all children had light infection intensities of hookworm (100%) and most had light infection intensities of *T. trichiura* (99.4%) and *A. lumbricoides* (91.9%). The remaining children had medium infection intensities; none of the children exhibited a heavy infection.

Table 19. Observed and estimated ‘true’ prevalence of helminth infections among school children in Chaani and Kinyasini in Unguja, Zanzibar in June/July 2007, and sensitivity of diagnostic methods/method combinations in relation to sampling effort.

Characteristics	<i>A. lumbricoides</i>		<i>T. trichiura</i>		Hookworm		<i>S. stercoralis</i>	
	Kato-Katz method		Kato-Katz method		Kato-Katz plus Koga agar plate method		Koga agar plate plus Baermann method	
	No. of children	Positive (%)	No. of children	Positive (%)	No. of children	Positive (%)	No. of children	Positive (%)
≥ 2 stool samples analyzed	367		367		365		363	
Cumulative positive result after analysis of								
1 st stool sample	52	14.2	94	25.6	43	11.8	20	5.5
2 nd stool sample	60	16.3	143	39.0	69	18.9	30	8.3
2 nd and/or 3 rd stool sample	62	16.9	171	46.6	79	21.6	37	10.2
Estimated ‘true’ prevalence (SD)	17.1% (4.0%)		48.4% (5.5%)		23.1% (4.7%)		14.4% (6.0%)	
Sensitivity of method (2 samples)	98.9%		96.3%		93.6%		70.7%	
Sensitivity of method (1 sample (SD))	89.3% (6.2%)		80.8% (5.1%)		74.6% (8.7%)		45.8% (17.9%)	

SD = standard deviation

15.4.3. Multiple helminth infections

In the final study cohort, 59.7% (216/362) of the children were infected with one or more helminth species. There were 126 children (34.8%) infected with a single, 56 children (15.5%) with 2, 29 children (8.0%) with 3 and 5 children (1.4%) with 4 different helminth species. Three-quarter of the children with a dual helminth infection harbored *A. lumbricoides* and *T. trichiura* concurrently.

15.4.4. Comparison with data obtained in 1994

Key results of the study from Marti and colleagues (Marti et al., 1996) carried out in 1994, are summarized in Table 20.

Comparing the overall prevalence of soil-transmitted helminth infections observed in 1994 with 2007, there was a drop of 39.6% (from 98.9% (95% CI: 98.3-99.5%) in 1994 to 59.7% (95% CI: 54.6-64.7%) in 2007). When the same sampling effort and diagnostic approach for species-specific helminths were considered, we found a reduction in the prevalence of *S. stercoralis* by 81.0% (prevalence in 1994 dropped from 34.8% to 6.6% in 2007). The respective prevalence drop for hookworm was 80.5% (from 93.9% to 18.3%), that for *A. lumbricoides* was 70.6% (from 57.5% to 16.9%), whereas *T. trichiura* prevalence decreased by 48.6% (from 90.6% to 46.6%). The infection intensities of all helminth species were significantly lower in 2007 than in 1994. The geometric mean infection intensity declined from 555 EPG to 1 EPG for hookworm (-99.8%), from 250 EPG to 5 EPG for *T. trichiura* (-97.9%), from 100 EPG to 2 EPG for *A. lumbricoides* (-97.9%) and the count of *S. stercoralis* larvae dropped from 3.7 to 0.05 larvae (-98.7%).

Table 20. Characteristics of helminth infections in children from Chaani and Kinyasini in Unguja, Zanzibar, as determined with the Kato-Katz method (*A. lumbricoides*, hookworm and *T. trichiura*) and the Baermann technique (*S. stercoralis*) in 1994 and 2007.

Year	Parasite species	Method	No. of children infected/ examined	Prevalence (95% CI)	Geometric mean of EPG* or larvae** (95% CI)	Maximal EPG* or larvae**	No. (%) of infected children stratified by infection intensity***		
							Light	Moderate	Heavy
1994	<i>A. lumbricoides</i>	Kato-Katz	685/1192	57.5 (54.7, 60.3)	99.8 (78.7, 126.5)	84720	406 (59.3)	271 (39.6)	8 (1.2)
	<i>T. trichiura</i>	Kato-Katz	1080/1192	90.6 (88.9 92.3)	250.1 (221.4, 283.4)	43248	793 (73.4)	280 (26.0)	7 (0.7)
	Hookworm	Kato-Katz	1119/1192	93.9 (92.5, 95.2)	554.7 (492.1, 625.3)	32064	809 (72.3)	172 (15.4)	138 (12.3)
	<i>S. stercoralis</i>	Baermann	419/1204	34.8 (32.1, 37.5)	3.7 (3.3, 4.1)	660	ND	ND	ND
2007	<i>A. lumbricoides</i>	Kato-Katz	62/367	16.9 (13.1, 20.7)	2.1 (1.4, 3.1)	17520	57 (91.9)	5 (8.1)	0
	<i>T. trichiura</i>	Kato-Katz	171/367	46.6 (41.5 51.7)	5.2 (4.0, 6.7)	2880	170 (99.4)	1 (0.6)	0
	Hookworm	Kato-Katz	67/367	18.3 (17.4, 25.9)	1.1 (0.8, 1.5)	2400	67 (100)	0	0
	<i>S. stercoralis</i>	Baermann	24/364	6.6 (7.1, 13.3)	0.05 (0.02, 0.07)	24	ND	ND	ND

CI: confidence interval; ND: not determined; * EPG: eggs per gram of stool, as determined by Kato-Katz method; ** Number of *S. stercoralis* larvae, as determined by Baermann examination; *** WHO classification: the thresholds for moderate and heavy infections are 5000 and 50,000 EPG for *A. lumbricoides*, 1000 and 10,000 EPG for *T. trichiura*, and 2000 and 4000 EPG for hookworm, respectively.

15.4.5. Results from univariate and multivariate analyses

The results from the univariate and multivariate analyses focusing on the final cohort of 362 school children and investigating the effect of age, sex and school location on helminth infection are shown in Table 21. Children in Chaani were at a significantly lower risk of a *T. trichiura* infection than pupils attending Kinyasini school, using univariate analysis (OR = 0.39, 95% CI: 0.25-0.59; $p = 0.001$). Adjustment for age and sex in multivariate analysis did not change this result (OR = 0.37, 95% CI: 0.24-0.57; $p = 0.001$). Children in Chaani were at a significantly lower risk of a hookworm infection than their counterparts in Kinyasini, both in univariate (OR = 0.52, 95% CI: 0.31-0.88; $p = 0.013$) and multivariate analyses (OR = 0.50, 95% CI: 0.29-0.86; $p = 0.011$). Boys were at a 2.6-fold higher risk of a hookworm infection than girls, both considering univariate (OR = 2.55, 95% CI: = 1.52-4.25; $p = 0.001$) and multivariate analyses (OR = 2.63, 95% CI: 1.56-4.43; $p = 0.001$).

No significant association of *A. lumbricoides* and *S. stercoralis* infections with sex, school location or age was observed, and there was no linear relationship between the EPG of either helminth species and the different school grades (data not shown).

Table 21. Association of exposure and infection with soil-transmitted helminths in 362 school children from Chaani and Kinyasini, Unguja, Zanzibar

Variable	Univariate model Association with infection				Multivariate model Association with infection			
	Odds ratio	(95% CI)	χ^2 (1 df)	P-value	Odds Ratio	(95% CI)	χ^2 (3 df)	P-value
n = 362								
<i>A. lumbricoides</i>							1.29	0.732
Sex (baseline = female)								
Male (42.3%)	1.35	(0.78, 2.34)	1.14	0.286	1.34	(0.77, 2.32)		
School (baseline = Kinyasini)								
Chaani (47.0%)	1.07	(0.62, 1.85)	0.06	0.805	1.08	(0.62, 1.87)		
Age (continuous)	1.02	(0.90, 1.16)	0.11	0.738	1.02	(0.90, 1.16)		
<i>T. trichiura</i>							23.24	0.001*
Sex (baseline = female)								
Male (42.3%)	1.38	(0.91, 2.09)	2.23	0.135	1.45	(0.94, 2.24)		
School (baseline = Kinyasini)								
Chaani (47.0%)	0.39	(0.25, 0.59)	19.68	0.001*	0.37	(0.24, 0.57)		
Age (continuous)	0.99	(0.90, 1.08)	0.09	0.760	0.95	(0.86, 1.05)		
Hookworm							19.98	0.001*
Sex (baseline = female)								
Male (42.3%)	2.55	(1.52, 4.25)	13.06	0.001*	2.63	(1.56, 4.43)		
School (baseline = Kinyasini)								
Chaani (47.0%)	0.52	(0.31, 0.88)	6.18	0.013*	0.50	(0.29, 0.86)		
Age (continuous)	1.04	(0.93, 1.17)	0.44	0.509	1.01	(0.90, 1.14)		
<i>S. stercoralis</i>							4.83	0.185
Sex (baseline = female)								
Male (42.3%)	1.70	(0.86, 3.36)	2.32	0.128	1.73	(0.87, 3.45)		
School (baseline = Kinyasini)								
Chaani (47.0%)	0.58	(0.29, 1.18)	2.35	0.125	0.57	(0.28, 1.16)		
Age (continuous)	1.01	(0.86, 1.18)	0.01	0.921	0.99	(0.84, 1.157)		

CI: confidence interval; df: degrees of freedom; * = significant p-values ($p \leq 0.05$).

15.5. Discussion

In Zanzibar, preventive chemotherapy targeting helminth infections has been implemented for over a decade now. The large-scale administration of anthelmintic drugs to school-aged children and other high-risk groups successfully reduced the morbidity due to helminthic diseases (Renganathan et al., 1995; Mohammed et al., 2008). The present study confirms that the prevalence and intensity of soil-transmitted helminth infections, including *S. stercoralis*, decreased sharply among school children in Unguja, in two settings where anthelmintic drugs have been administered on a fairly regular schedule. Indeed, whilst we found 40.3% of the 362 school children with complete data records to be free of any soil-transmitted helminth infection, in 1994, only 1.1% of more than 1200 school children were considered non-infected. Moreover, most of the infected children from our study harbored only one helminth species (58.3%), and infections were primarily of light intensity, which is different to the situation in 1994 (Marti et al., 1996).

It is important to note that the prevalence of *T. trichiura* (46.6%), hookworm (21.6%), *A. lumbricoides* (16.9%) and *S. stercoralis* (10.2%) in 2007 was determined on the basis of at least 2 stool samples examined from each child using different methods, whereas in 1994 only a single stool sample per individual was subjected to the Kato-Katz method for the diagnosis of common soil-transmitted helminths and the Baermann method for *S. stercoralis*. Hence, the diagnostic approach adopted in the current study was more sensitive than the one in 1994. It is therefore conceivable that the reported helminth prevalence in 1994 was an underestimation of the 'true' situation at the time, and hence the calculated reduction in prevalence of helminth infections might be higher than reported here. The significant reduction in the prevalence and the decline in infection intensity by > 95% of each soil-transmitted helminth infection are encouraging. Indeed, these findings are likely the result of regular administration of anthelmintic drugs. Additionally, improvements in water supply and sanitation and targeted health education since 1994 might have contributed to lowering soil-transmitted helminth infections. However, no related data were collected in 1994 and 2007 and as discussed below, the sanitary infrastructure was deemed to be still inadequate in 2007. It should be noted, however, that the overall prevalence of soil-transmitted helminth infections in 2007, approximately 6 months after the most recent treatment round, remains above 50% in children attending the schools of Kinyasini and Chaani. Hence, according to WHO guidelines (Montresor et al., 1998), annual treatment with anthelmintic drugs is still warranted. Of note, the 'real' helminth prevalence among school-aged children might be higher, since some

children do not attend school in Zanzibar (primary school enrollment in Zanzibar was 97.8% in 2006 (Worldbank, 2006)). It is conceivable that these non-attendees are at a higher risk of soil-transmitted helminth infections.

The observed prevalence of helminth infections in our study – with the exception of *A. lumbricoides* – increased by more than 80% when multiple stool samples per individual were examined and a combination of diagnostic methods considered. Underlying reasons, among others, include (i) low egg output due to light infection intensities, which was observed in most of the infected children, (ii) day-to-day variation in egg output, and (iii) lack of sensitivity of the diagnostic assays (Hall, 1981; Booth et al., 2003; Knopp et al., 2008). Areas subjected to large-scale anthelmintic drug administrations are characterized by low helminth infection intensities, which call for diagnostic tests with a high sensitivity (Steinmann et al., 2007; Bergquist et al., 2009; Knopp et al., 2009; Stothard et al., 2009c). Examination of repeated stool samples increases diagnostics sensitivity (de Vlas and Gryseels, 1992; Utzinger et al., 2001; Knopp et al., 2008; Steinmann et al., 2008), but this approach raises concerns about compliance. In our study, however, over 90% of the children supplied at least 2 stool samples of sufficient quantity to perform a suite of diagnostic tests.

School children in Kinyasini had a higher risk of *T. trichiura* and hookworm infections than those from Chaani. Local risk factors sustaining helminth transmission can include specific environmental differences, such as soil-composition and moisture, as well as socioeconomic discrepancies and differences in sanitation, hygiene and health seeking behavior (de Silva et al., 2003; Mabaso et al., 2003; Brooker et al., 2004; Raso et al., 2005; Raso et al., 2006; Stothard et al., 2008). The lack of detailed appraisal of some of these risk factors is a limitation of the present study and is the focus of our ongoing work. The observed higher risk for boys to be infected with hookworm coincides with findings from Pemba and elsewhere in Africa, and might result from behavioral, immunological or genetic idiosyncracies (Bradley et al., 1992; Albonico et al., 1997b; Stoltzfus et al., 1997; Raso et al., 2006). Age showed no significant association with infection prevalence of any helminth species and the school grade showed no association with infection intensity. This implies that older children in higher grades were not better off due to multiple rounds of school-based treatments than their younger counterparts who were only treated once or twice before our survey. These findings are in contrast to a previous study conducted in Pemba, where lower mean infection intensities with *A. lumbricoides*, hookworm and *T. trichiura* in children of grade 5 compared to children of grade 1 were explained as a beneficial effect of regular treatment (Albonico et al., 2003). However, in the Pemba study, children from grade 1 had not

been treated before, whereas in our study the youngest children were 7 years old and thus had most likely experienced at least one round of mass-drug administration in the frame of the GPELF, which distributed albendazole plus ivermectin to the whole eligible population above the age of 5 years (Mohammed et al., 2006). Additionally, all children who participated in our study in June/July 2007 were supposed to have been treated in their schools in December 2006. However, due to an interruption in drug distributions in the two schools in 2001-2003, children in higher grades were unlikely to have experienced more than 4 rounds of school-based treatment (2003-2006).

In the present study, 59.7% of the children were infected with soil-transmitted helminths, primarily at low intensity, although they had been subjected, most likely, to 2 or even more rounds of anthelmintic drug administration in the frame of school-based treatment campaigns and the GPELF. The following reasons are offered for consideration why the deworming interventions were not more effective in terms of prevalence reduction. First, infections with *T. trichiura* count for the largest part of this high overall helminth prevalence. Indeed, the prevalence of *T. trichiura* in mid-2007 was still 46.6% and the relatively smaller decrease in prevalence (reduction of 48.6%) compared to *A. lumbricoides*, hookworm and *S. stercoralis* (reductions of over 70%), confirms the reported low efficacy of the benzimidazoles albendazole and mebendazole against *T. trichiura* (Marti et al., 1996; Keiser and Utzinger, 2008; Stothard et al., 2009b). Conflicting results have been reported regarding the efficacy of an albendazole-ivermectin combination against *T. trichiura* infections in the frame of the GPELF (Olsen, 2007; Ndyomugenyi et al., 2008). Hence, new research is needed to determine the efficacy and safety of new drugs and combination of existing drugs targeting *T. trichiura*. Second, children not attending school or those missing the day of treatment, together with pre-school children might contribute substantially to the overall transmission of soil-transmitted helminths, which leads to rapid re-infection of children who have been dewormed successfully. Third, insufficient knowledge on soil-transmitted helminth transmission might result in risky behavior (e.g., eating unpeeled fruits or walking barefoot) and (un)intentional pollution of the environment abetted by a lack of sanitary infrastructures and access to clean water. Hence, to render preventive chemotherapy campaigns more effective, regular informal meetings of health professionals, community leaders and headmasters must be organized to keep awareness high. Regular teaching and training lessons for locally involved health staff (e.g., health teachers and village health workers) must be held to guarantee knowledge transfer to villagers and school children, and hence to change behavior of high-risk groups. Periodic de-worming campaigns in schools and whole communities could

be used not only for drug administration, but could go hand-in-hand with focussed health education on how to prevent worm infection and transmission. Preventive chemotherapy coupled with 'preventive behavior' might, however, only reduce soil-transmitted helminth transmission successfully if they are complemented by improvements in water and sanitation. At present, children attending Chaani and Kinyasini schools have reasonable access to well or tap water at home (Stothard et al., 2002), but only a small proportion of households has latrines and there is no communal system for solid waste management. These contextual factors might explain why the prevalence of soil-transmitted helminth infections in Unguja is still high, coupled with the risk of rapid 're-worming' after successful de-worming.

We conclude that the national helminth control programs in Zanzibar have been successful in reducing both prevalence and intensity of soil-transmitted helminth infections, and hence morbidity and transmission have likely decreased. However, similar to other large-scale and long-term helminth control programs, for example the one in China (Hotez et al., 1997), efforts to control intestinal nematode infections with conventional chemotherapeutic agents may not be sufficient to yield a lasting reduction in prevalence and intensity. Therefore, it will be important to find out about the needs of educational, behavioral and environmental changes in Zanzibar to specifically target future health interventions. From a more global perspective, the observations on Zanzibar represent particularly pertinent information for public health strategists developing disease forecasting predictions in the face of ongoing de-worming initiatives in various endemic countries. Through a combination of pragmatic field research and application of epidemiological models it should be possible to predict the true impact of chemotherapy on several soil-transmitted helminth infections simultaneously. By setting clear end-point targets in terms of prevalence and intensity of infection, better monitoring and evaluation of programs seems in reach.

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16. Albendazole and mebendazole administered alone or in combination with ivermectin against *Trichuris trichiura*: a randomized controlled trial

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16.1. Abstract

Background. Single-dose albendazole and mebendazole show limited efficacy in the treatment of trichuriasis. The combination of albendazole with ivermectin improves efficacy, but a mebendazole-ivermectin combination has not been investigated before.

Methods. We carried out a randomized controlled trial in two schools in Zanzibar, Tanzania, to assess the efficacy and safety of albendazole (400 mg) plus placebo, albendazole plus ivermectin (200 µg/kg), mebendazole (500 mg) plus placebo, and mebendazole plus ivermectin in children with a parasitologically confirmed *Trichuris trichiura* infection. Cure rate (CR) and egg reduction rate (ERR) were assessed by intention-to-treat analysis. Adverse events (AEs) were monitored within 48 h post-treatment.

Results. Complete data records were available for 548 children. The highest CR against *T. trichiura* was achieved with a mebendazole-ivermectin combination (55%). Low CR were observed with albendazole-ivermectin (38%), mebendazole (19%) and albendazole (10%). Compared to placebo, the use of ivermectin statistically significantly increased the CRs from 14% to 47% (odds ratio: 0.19, 95% confidence interval (CI): 0.12-0.28). The highest ERR (97%; 95% CI: 95-98%) was observed using the mebendazole-ivermectin combination, followed by albendazole-ivermectin (91%, 95% CI: 87-94%), mebendazole (67%, 95% CI: 52-77%) and albendazole (40%, 95% CI: 22-56%). AEs, reported by 136 children, were generally mild, with no significant difference between treatment arms.

Conclusions. Addition of ivermectin improves the therapeutic outcomes of both albendazole and mebendazole against *T. trichiura* and might be considered for use in soil-transmitted helminth control programs and individual patient management.

Clinical trial registration. Current Controlled Trials identifier: ISRCTN08336605.

Keywords: Soil-transmitted helminthiasis, *Trichuris trichiura*, treatment, randomized controlled trial, Zanzibar

16.2. Introduction

An estimated 604-795 million people are infected with the whipworm *Trichuris trichiura*, causing a global burden of 6.4 million disability-adjusted life years lost (Chan, 1997; Bethony et al., 2006; Hotez and Kamath, 2009). The current strategy against *T. trichiura* and other soil-transmitted helminth infections (*Ascaris lumbricoides* and hookworm) is to regularly administer anthelmintic drugs, mainly albendazole and mebendazole, to school-aged children and other at-risk populations (WHO, 2006; Smits, 2009). While both drugs show good therapeutic profiles against *A. lumbricoides*, mebendazole fails to effectively clear hookworm infections, and neither drug is satisfactory against *T. trichiura*. In a meta-analysis of randomized, placebo-controlled trials, the average cure rate (CR) of single-dose albendazole (400 mg) against *T. trichiura* was reported to be only 28%. Mebendazole (500 mg), with an average CR of 36%, performed slightly better (Keiser and Utzinger, 2008). Improved treatment outcomes against *T. trichiura* were observed when using triple-dose treatments of either drug (Keiser and Utzinger, 2010; Steinmann et al., 2010). Albendazole and mebendazole can be co-administered with other deworming drugs, foremost is the combination of albendazole with ivermectin (200 or 400 µg/kg) as used against lymphatic filariasis. This combination therapy showed promising results against *T. trichiura*; observed CRs were 65% and 80% in two trials (Beach et al., 1999; Belizario et al., 2003). However, a third trial found no effect by adding ivermectin to albendazole (Ndyomugenyi et al., 2008). Despite the somewhat higher efficacy of mebendazole in comparison to albendazole against trichuriasis, the efficacy and safety of a mebendazole-ivermectin combination has yet to be investigated (Olsen, 2007; Keiser and Utzinger, 2010).

In Zanzibar, control programs have significantly reduced the prevalence and intensity of soil-transmitted helminthiasis (Knopp et al., 2009). However, in contrast to *A. lumbricoides* and hookworm, the prevalence of *T. trichiura* infections has remained high (Knopp et al., 2008; Knopp et al., 2009). Against this background, we were motivated to assess the efficacy and safety of albendazole and mebendazole combined with ivermectin against *T. trichiura* and to compare treatment outcomes with standard monotherapies.

16.3. Patients and methods

16.3.1. Study area and population

The study was conducted in Unguja, Zanzibar Island, Tanzania between March and May 2009. Stool samples were collected from children attending the schools in Kinyasini and Kilombero, where soil-transmitted helminthiasis is highly endemic (Knopp et al., 2009). Both schools are located in district “North A”, 30-40 km northeast of Zanzibar Town.

16.3.2. Ethical considerations

Ethical approval was granted by the Ethics Committee of Basel, Switzerland (no. 13/09) and the Ministry of Health and Social Welfare, Zanzibar (no. ZAMEC/0001/09). The trial was registered with Current Controlled Trials (identifier: ISRCTN08336605). Written informed consent was obtained from parents or guardians of participating children, whereas children consented orally. Participation was voluntary, and individuals could withdraw from the trial at any time.

16.3.3. Eligibility criteria for participants

Children attending grades 1-7 in the two schools were eligible, if they met all of the following inclusion criteria: written informed consent provided by parents/guardian; age ≥ 5 years; sufficiently large stool sample to perform duplicate Kato-Katz thick smears at baseline survey; infection with *T. trichiura*; and submission of second stool sample subjected to duplicate Kato-Katz thick smears prior to treatment.

After randomization, children were excluded from treatment if fulfilling any of the following exclusion criteria: for females, pregnant, as verbally assessed by medical personnel; presence of systemic illnesses (e.g., fever, severe illness); and anthelmintic treatment within the last 4 weeks.

16.3.4. Sample size

Sample size calculation was based on the range of CRs of albendazole and mebendazole (20-45%) vs. albendazole plus ivermectin (65-80%) against *T. trichiura* according to recent reviews (Olsen, 2007; Keiser and Utzinger, 2008). Using a significance level of 5% to detect greater efficacy of combination therapy, a power of 80%, and an equation by Fleiss (1981)

(Fleiss, 1981), the minimum required number of individuals per treatment group at different CRs was 105.

The prevalence of *T. trichiura* infections was conservatively estimated to be 30% (Knopp et al., 2009), and the compliance for stool submission was assumed to be 90% per sample. Hence, we estimated that ~2000 subjects were needed to identify 600 *T. trichiura*-infected individuals. However, the baseline survey revealed a prevalence of *T. trichiura* of >50% in both schools. Hence, we stopped enrolling after 1240 children had been invited.

16.3.5. Baseline parasitological survey

First, the name, age, sex, and school grade of each child were recorded. Next, children received containers with unique IDs, and were invited to bring a fresh stool sample the following morning. Within 2 weeks a total of 1240 children were invited to participate. Children with a microscopically-confirmed *T. trichiura* infection were asked for a second stool sample and their body weight was taken.

16.3.6. Randomization

The trial statistician was provided with the list of IDs of 618 *T. trichiura*-positive children and generated a computer-based random allocation sequence (numbers 1-4). The numbers were decoded for each school by one of two researchers (SK for Kilombero, and BS for Kinyasini) to assign children either to albendazole (400 mg; Laboratoria Wolfs) plus placebo (Hermes Edulcorants), albendazole plus ivermectin (200 µg/kg; Merck & Co.), mebendazole (500 mg; Janssen-Cilag) plus placebo, or mebendazole plus ivermectin. Trial medications were prepared in identical envelopes labeled with unique IDs and sealed. As ivermectin is administered according to patient weight, ivermectin and placebo tablets were counted and packed according to children's weight.

On the day of treatment, eligible children were examined by medical personnel for exclusion criteria before drug administration. Administrators opening the sealed envelopes and administering the drugs were “blinded” to group assignment. However, to the trained eye, the gravure on the albendazole or mebendazole tablets was not identical, and placebos were slightly smaller than ivermectin tablets. Children did not have sight of the tablets since drug administrators placed the tablets directly in their mouth, but differences in palatability and taste of the tablets might exist. Drugs were swallowed with clean water and accompanied by a small

food item. A clinician was present and monitored acute adverse events (AEs) and provided medical assistance if necessary. The staff of the nearby health centers had undergone pharmacovigilance training. The medical staff was informed about the date of treatment and the health centers were provided with first aid drugs (oral and intravenous analgesics and anti-anaphylactics) and remained open for 24 h following treatment. Children, parents and teachers were advised to refer to these health centers in case of AEs.

All laboratory personnel, including the outcome assessors, were blinded to group assignment. Only the data examiners saw unblinded data and one of them (JH) had no contact with the study participants although the success of the blinding was not assessed formally.

16.3.7. Assessment of adverse events

48 h post-treatment, AEs due to the treatment were assessed by a pre-tested questionnaire. Children were interviewed by trained personnel of the Helminth Control Laboratory Unguja (HCLU), who were familiar with potential AEs resulting from anthelmintic treatment. Solicited AEs were headache, vertigo, allergic reactions (pruritus, urticaria, and anaphylaxis), shivering, abdominal cramps, nausea, vomiting, diarrhea, and fever. AEs were recorded and graded as follows: *mild*: not requiring any intervention; *moderate*: requiring medication for symptomatic relief upon request, or present and interfering with normal daily activities; and *severe*: requiring medical intervention beyond symptomatic relief.

16.3.8. Follow-up

Three weeks after treatment, study participants were asked for two consecutive stool samples. Approximately 120 children were enrolled daily and the procedure was continued for 3 weeks until, in mid-May 2009, the large majority of treated children had provided two stool samples for follow-up.

16.3.9. Laboratory procedures

Stool samples were transferred to HCLU and processed the same day, adhering to standardized, quality-controlled methods (Knopp et al., 2010). Duplicate 41.7 mg Kato-Katz thick smears were prepared (Katz et al., 1972). Slides were quantitatively examined under a microscope for the presence of hookworm eggs after a clearing time of 20-40 min, and for *T. trichiura* and *A. lumbricoides* eggs a few hours later.

16.3.10. Primary and secondary outcomes

Primary outcome measures were the CR and ERR achieved by treatment with any drug regimen against *T. trichiura* infections. Secondary outcome measures were the frequencies of AEs. The CR was determined as the percentage of children excreting eggs before treatment who became negative after treatment. The ERR was calculated as the reduction in the groups geometric mean (GM) egg count, including infected and non-infected subjects at follow-up, according to World Health Organization (WHO) guidelines (Montresor et al., 1998). Confidence intervals (CIs) for the ERR were calculated, using a bootstrap re-sampling method with 2000 replicates.

16.3.11. Statistical analysis

An intention-to-treat analysis was pursued. All individuals with primary endpoint data records were included in the final analyses (see Figure 1). The species-specific numbers of helminth eggs recorded from 4 Kato-Katz slides before and after treatment were added and multiplied by a factor 6 to obtain the arithmetic mean (AM) of the eggs per gram of stool (EPG) for each individual. AMs were used to determine whether the intensity of infection with soil-transmitted helminths was light, moderate, or heavy, using WHO cut-offs, and to calculate the GM infection intensities as a summary measure among the treatment groups (Montresor et al., 1998).

The 2x2 factorial design enabled the determination of the treatment efficacy of the 4 regimens against *T. trichiura*, of potential interactions between the interventions and of the interventions by school, using regression analysis. Odds ratios (ORs) and respective *P*-values were used to explain differences between the 4 treatment groups. Significance was given at $P < .05$.

Differences in the median time (days) from treatment to the last collected stool sample at follow-up between the schools and the 4 treatment groups were assessed using the Wilcoxon test. Drug-related AEs were analyzed using ordinal logistic regression with the untoward effect classified as absent, mild, moderate, or severe and the factorial treatment regimens (without interaction term) as predictor variables.

Data were double-entered and discrepancies removed in tracing back to original records. Statistical analyses were performed using STATA version 10 (StataCorp LP; College Station, USA), bootstrap CIs were calculated using R 2.9.1 (R Development Core Team; Vienna, Austria).

16.4. Results

16.4.1. Study Cohort

Among 1240 children invited for the baseline screening, 174 did not participate, and 456 were excluded (Figure 19). The remaining 610 children were randomly assigned to one of the 4 treatment arms. An additional 8 children were wrongly included in the randomization, although they were not infected with *T. trichiura* (n=2), or had incomplete parasitological data (n=6). These children were excluded from subsequent analyses. Thirty-eight children did not receive the assigned intervention because they were either absent during the treatment (n=36) or met one of the exclusion criteria at the day of treatment (n=2). Twenty-four children were lost to follow-up. Complete data records for the final analysis were available for 548 children.

AEs were determined from 564 among the 572 treated children (99%).

16.4.2. Baseline Parasitological Survey

Overall, 1066 children submitted a stool sample for Kato-Katz examination at baseline. The prevalence of *T. trichiura* was 63%. Hookworm and *A. lumbricoides* infections were diagnosed in 20% and 9% of the children, respectively.

16.4.3. Baseline Characteristics

The mean age of the 610 randomized children was 11 years (Table 22). Children's mean weight was similar in all groups (range: 29.4-30.6 kg). The proportion of girls differed slightly among groups (range: 47-60%). The GM of *T. trichiura* infections were similar in the 4 treatment groups (121-154 EPG), and infection intensities were mainly light (92-94%).

The proportion of children co-infected with hookworm (range: 24-29%), and the intensity of hookworm infection (range: 60-66 EPG) was similar in all groups. Concurrent infections with *A. lumbricoides* were found in 10-13%; the GM was highest in the albendazole-placebo group (3401 EPG) and lowest in the mebendazole-ivermectin group (381 EPG).

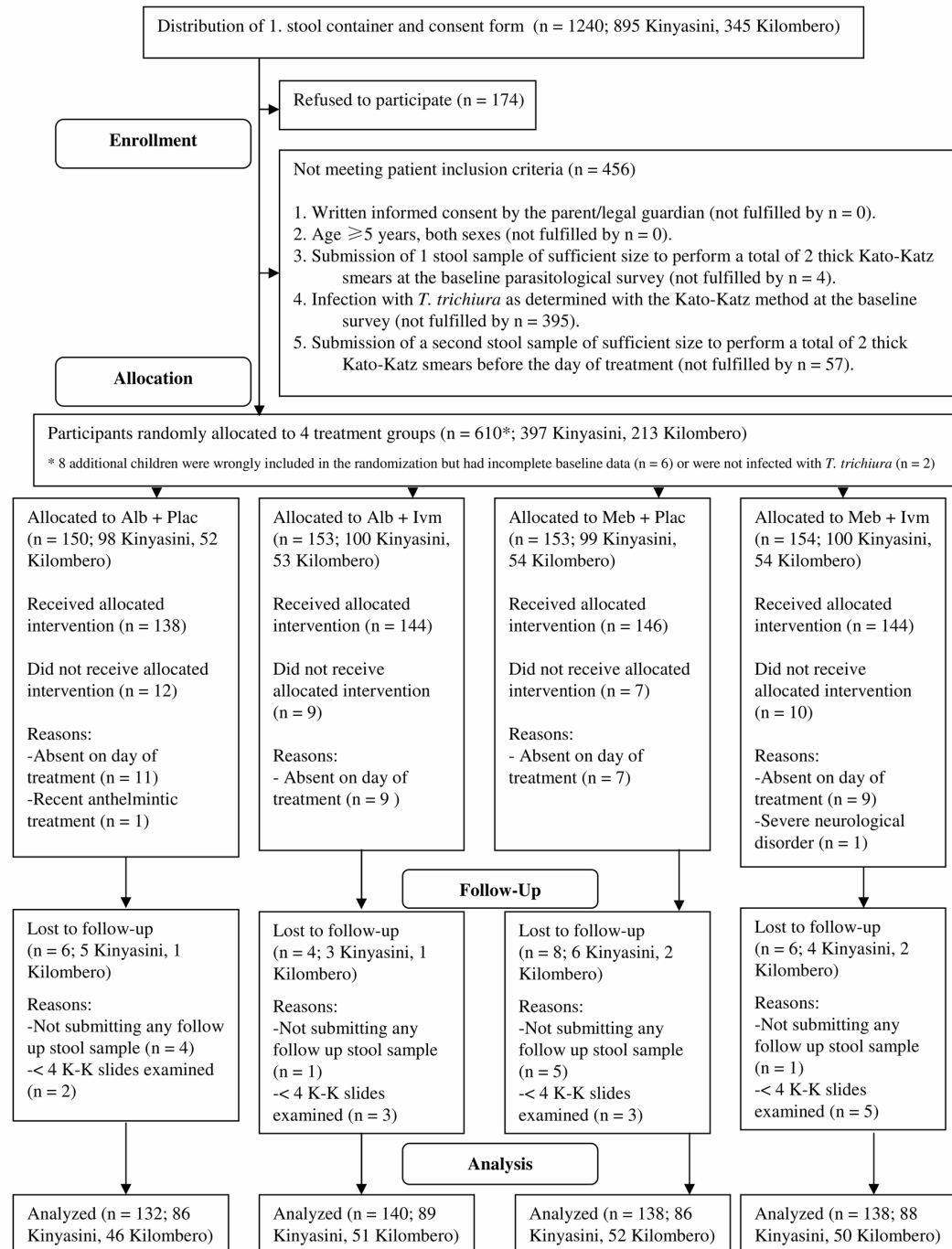


Figure 19. Flow diagram of the randomized controlled trial comparing the efficacy and safety of albendazole and mebendazole alone and in combination with ivermectin against *T. trichiura* in children from primary schools in Kinyasini and Kilombero on Unguja Island, Zanzibar, in early 2009.

Table 22. Baseline demographic and clinical characteristics of 610 children included in the randomized controlled trial conducted in early 2009 in the primary schools of Kilombero and Kinyasini on Unguja Island, Zanzibar, Tanzania.

Characteristic	Albendazole plus placebo (n=150)	Albendazole plus ivermectin (n=153)	Mebendazole plus placebo (n=153)	Mebendazole plus ivermectin (n=154)
Mean age \pm SD, years	10.9 \pm 2.6	11.0 \pm 2.8	10.8 \pm 2.8 (152 observations ^a)	11.0 \pm 2.6
No. of females/ no of males	71/79	84/69	89/64	92/62
No. of participants at Kilombero/Kinyasini	52/98	53/100	54/99	54/100
Mean weight \pm SD, kg	30.5 \pm 9.9 (145 observations ^a)	30.6 \pm 10.2 (151 observations ^a)	29.5 \pm 9.2 (149 observations ^a)	29.4 \pm 8.9 (151 observations ^a)
<i>Trichuris trichiura</i> , n (%)	150 (100)	153 (100)	153 (100)	154 (100)
Geometric mean EPG	154	121	136	154
Median (25-75%)	156 (66-302)	108 (54-276)	126 (60-282)	159 (78-273)
Infection intensity n (%)				
Light	138 (92.0)	142 (92.8)	144 (94.1)	144 (93.5)
Moderate	12 (8.0)	11 (7.2)	9 (5.9)	10 (6.5)
Heavy	0	0	0	0
<i>Ascaris lumbricoides</i> , n (%)	19 (12.7)	15 (9.8)	19 (12.4)	20 (13.0)
Geometric mean of positive EPG	3401	1839	2601	381
Median (25-75%)	4584 (1122-17,772)	2904 (774-6846)	11394 (1104-23,394)	603 (17-8183)
Infection intensity n (%)				
Light	11 (57.9)	10 (66.7)	8 (42.1)	14 (70.0)
Moderate	7 (36.8)	4 (26.7)	11 (57.9)	5 (25.0)
Heavy	1 (5.3)	1(6.6)	0	1 (5.0)
Hookworm , n (%)	44 (29.3)	37 (24.2)	39 (25.5)	40 (26.0)
Geometric mean EPG	60	66	65	64
Median (25-75%)	60 (18-152)	66 (36-132)	48 (18-192)	51 (18-263)
Infection intensity n (%)				
Light	44 (100)	36 (97.3)	39 (100)	40 (100)
Moderate	0	1 (2.7)	0	0
Heavy	0	0	0	0

16.4.4. Time difference for follow-up

Stool samples for follow-up were collected between 22 and 39 days post-treatment. The median follow-up period was 23 days in Kilombero and 30 days in Kinyasini ($P<.001$). The median follow-up period for the 4 treatment groups was 29 days for all groups ($P=.954$).

16.4.5. CR and ERR against *T. trichiura*

By design, all children included in the primary analysis were infected with *T. trichiura* before treatment. The highest CR (55%) was achieved with a mebendazole-ivermectin combination, followed by albendazole-ivermectin (38%). Particularly low CRs were achieved when administering albendazole and mebendazole alone, 10% and 19%, respectively (Table 23).

Since there was no evidence of a statistically significant interaction between the effects of the medications (OR=1.03, 95% CI: 0.43-2.44; Table 24), the effect of each drug on *T. trichiura* can be interpreted individually according to the results of the primary analysis. Mebendazole cured statistically significantly more *T. trichiura* than albendazole (OR=2.05, 95% CI: 1.38-3.04), and ivermectin cured significantly more *T. trichiura* than placebo (OR=5.40, 95% CI: 3.55-8.22). Hence, ivermectin has an additive effect on both albendazole and mebendazole. After adjusting for sex, age, and days to last follow-up stool sample, none of the estimates changed significantly. The addition of ivermectin to albendazole or mebendazole improved the CR from 14% to 47% in comparison with placebo (Figure 20). Mebendazole, regardless of whether combined with ivermectin or placebo, showed a higher CR than the albendazole analogous treatments (37% vs. 24%).

The pattern observed for ERRs was similar to that for CRs (Table 23). Medication with mebendazole-ivermectin resulted in the highest ERR (97%; bootstrap 95% CI: 95-98%). Treatment with albendazole-ivermectin resulted in a statistically significant lower ERR (91%; 95% CI: 87-94%), as indicated by non-overlapping bootstrap CIs. Lowest ERRs were achieved when applying mebendazole (67%, 95% CI: 52-77%) or albendazole (40%; 95% CI: 22-56%) alone.

Table 23. Cure rate (CR) and egg reduction rate (ERR) of *T. trichiura* and infection characteristics of *A. lumbricoides* and hookworm following administration of albendazole and mebendazole given alone or in combination with ivermectin among 548 children from the primary schools of Kilombero and Kinyasini on Unguja Island, Zanzibar, Tanzania.

Confidence intervals (CI) of the ERRs have been constructed by a bootstrap re-sampling

Helminth	Characteristic	Albendazole placebo	plus	Albendazole ivermectin	plus	Mebendazole placebo	plus	Mebendazole ivermectin	plus
<i>T. trichiura</i>	Before treatment: positive	132		140		138		138	
	After treatment: still positive n (%)	119	(90.2)	87	(62.1)	112	(81.2)	62	(44.9)
	CR, %	9.8	(5.4-16.3)	37.9	(29.8-46.4)	18.8	(12.7-26.4)	55.1	(46.4-63.5)
	Geometric mean EPG before treatment	154		127		146		160	
	Geometric mean EPG after treatment	92		11		49		5	
	ERR (95% CI)	40.3	(21.5-55.7)	91.1	(87.2-94.0)	66.7	(52.3-76.9)	96.7	(95.0-97.9)
Hookworm	Before treatment: positive	39		30		34		35	
	After treatment: still positive n (%)	16	(41.0)	10	(33.3)	22	(64.7)	26	(74.3)
	Geometric mean EPG before treatment	67		74		61		58	
	Geometric mean EPG after treatment	4		3		13		29	
<i>A. lumbricoides</i>	Before treatment: positive	14		14		18		18	
	After treatment: still positive n (%)	0	(0)	1	(7.1)	4	(22.2)	0	(0)
	Geometric mean EPG before treatment	2680		1699		2414		553	
	Geometric mean EPG after treatment	0		1		5		0	

Table 24. Multiple regression analysis for the 2 x 2 factorial design to assess the risk of a persistent *T. trichiura* infection after treatment with (i) albendazole or mebendazole and (ii) ivermectin or placebo in 548 school children from the primary schools of Kilombero and Kinyasini on Unguja Island, Zanzibar, Tanzania.

Two-way interactions between the effects of the medications (interaction term: ivermectin x albendazole, ivermectin x mebendazole) and between the effects of schools and medications (interaction term: ivermectin x Kinyasini, albendazole x Kinyasini) were assessed. Outcome variable is a *T. trichiura* infection after treatment. Explanatory variables in brackets [vs. variable] indicate the baseline of comparison.

		OR	LCL	UCL	P
Primary analysis	n = 548				
	Albendazole [vs. mebendazole]	2.05	1.38	3.04	<0.001
	Placebo [vs. ivermectin]	5.40	3.55	8.22	<0.001
	Kinyasini [vs. Kilombero]	1.08	0.72	1.63	0.702
2-way analysis	n = 548				
	Albendazole [vs. mebendazole]	1.47	0.72	2.99	0.285
	Placebo [vs. ivermectin]	8.54	3.73	19.59	<0.001
	Kinyasini [vs. Kilombero]	1.08	0.57	2.07	0.806
	Ivermectin x mebendazole [vs. ivermectin x albendazole]	1.03	0.43	2.44	0.950
	Albendazole x Kinyasini [vs. albendazole x Kilombero]	1.66	0.72	3.83	0.236
	Ivermectin x Kinyasini [vs. ivermectin x Kilombero]	0.49	0.20	1.20	0.118
Adjusted analysis	n = 548				
	Albendazole [vs. mebendazole]	2.02	1.35	3.00	0.001
	Placebo [vs. ivermectin]	5.40	3.54	8.25	<0.001
	Kinyasini [vs. Kilombero]	0.98	0.54	1.78	0.951
	Female [vs. male]	0.68	0.45	1.02	0.059
	Age	0.97	0.90	1.05	0.407
	Days from treatment to last follow-up stool sample	1.01	0.96	1.07	0.642

OR: Odds ratio, LCL: lower confidence limit, UCL: upper confidence limit, P: p-value

16.4.6. Treatment outcome on hookworm and *A. lumbricoides*

Concurrent hookworm and *A. lumbricoides* infections were found in 25% and 12%, respectively, of the 548 children included in the primary analysis. The highest decreases in prevalence and intensity of hookworm infections were found within the albendazole treated groups (Table 23). No drug combination was superior to albendazole treatment against *A. lumbricoides*.

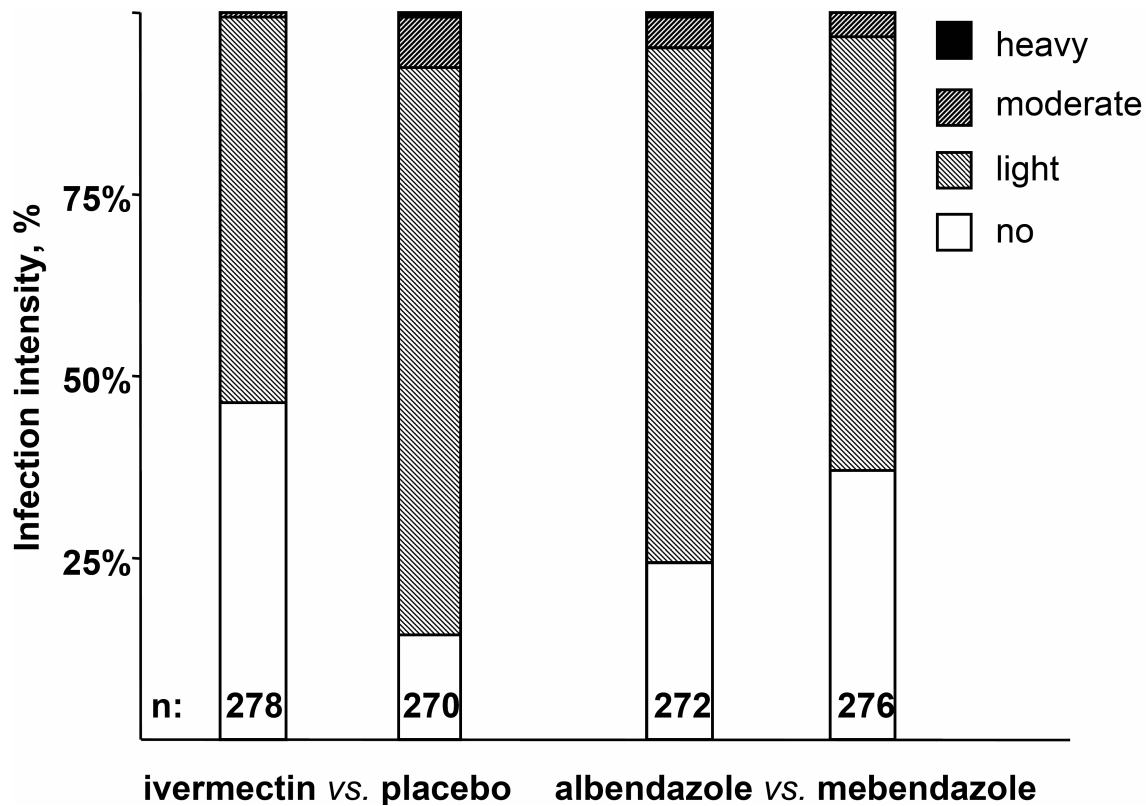


Figure 20. Independent treatment effects of albendazole vs. mebendazole and ivermectin vs. placebo on infection intensities of *T. trichiura* (administration of albendazole and mebendazole alone and in combination with ivermectin in a 2x2 factorial design).

Infection intensities were classified according to WHO cut-offs (Montresor et al., 1998). Intensity class “no” represents the cure rate (CR), “light” light infection intensities after treatment, “moderate” moderate infection intensities after treatment, “heavy” heavy infection intensities after treatment.

16.4.7. Adverse Events

Abdominal cramps were reported by 13% (72/564) of the participants, headache and fatigue by 5% (27/564), nausea by 5% (26/564), diarrhea and vertigo by 3% (19/564), and allergic reactions by 1.4% (8/564) (Table 25). Four children reported pruritus without rash, 4 had localized urticaria, and 6 reported shivering. Most AEs (72%; 166/229) were mild, the remaining 28% (63/229) were moderate. AEs were mainly self-limiting. Relief through drugs was sought by 29 children, and 3 used local remedies.

The proportion and intensity of abdominal cramps was slightly higher in the treatment regimes containing ivermectin, without statistical significance (OR=1.30; 95% CI: 0.79-2.14) (Table 25). Girls reported statistically significant less often vertigo than boys (OR=0.22; 95%

CI: 0.06-0.76). Older children were more likely to suffer from fatigue (OR=1.53; 95% CI: 1.04-2.26), but reported less vomiting (OR=0.71; 95% CI: 0.53-0.95) and diarrhea (OR=0.81; 95% CI: 0.67-0.98). Children with moderate *T. trichiura* infection intensities at baseline were more likely to report shivering than those with light infections (OR=6.76; 95% CI: 1.20-38.11).

Table 25. Adverse events (AEs) reported 48 h after treatment with albendazole or mebendazole in combination with ivermectin or placebo by school children from Kinyasini and Kilombero on Unguja island, Zanzibar (n=564).

Characteristic	Albendazole plus placebo (n=136)		Albendazole plus ivermectin (n=144)		Mebendazole plus placebo (n=143)		Mebendazole plus ivermectin (n=141)	
	AEs n (%)	Moderate intensity n	AE n (%)	Moderate intensity n	AEs n (%)	Moderate intensity n	AEs n (%)	Moderate intensity n
Abdominal cramps	15 (11.0)	3	21 (14.6)	12	17 (11.9)	3	19 (13.5)	6
Fatigue	8 (5.9)	NA	4 (2.8)	NA	6 (4.2)	NA	9 (6.4)	NA
Headache	8 (5.9)	5	5 (3.5)	3	7 (4.9)	1	7 (5.0)	2
Nausea	5 (3.7)	1	11 (7.6)	4	3 (2.1)	2	7 (5.0)	0
Diarrhea	6 (4.4)	0	4 (2.8)	2	5 (3.5)	1	4 (2.8)	0
Vertigo (dizziness)	6 (4.4)	2	2 (1.7)	2	6 (4.2)	1	5 (3.5)	0
Fever	4 (2.9)	1	6 (4.2)	3	4 (2.8)	2	2 (1.4)	0
Vomiting	3 (2.2)	0	3 (2.1)	1	3 (2.1)	0	0	0
Allergic reaction	2 (1.7) ^a	0	5 (3.5) ^{a, b}	3	1 (0.7) ^b	1	0	0
Shivering	3 (2.2)	0	3 (2.1)	2	0 (0.0)	0	0	0
Total (≥1)	60	12	64	32	52	11	53	8

^a Pruritus without rash

^b Localized urticaria

16.5. Discussion

We found low CRs with standard single-dose albendazole and mebendazole against *T. trichiura* infections among schoolchildren in Zanzibar, confirming results from a meta-analysis of randomized, placebo-controlled trials (Keiser and Utzinger, 2008). An albendazole-ivermectin combination resulted in a statistically significant higher CR against *T. trichiura*, hence supporting findings from two previous trials (Beach et al., 1999; Belizario et al., 2003). A mebendazole-ivermectin combination – used for the first time against *T. trichiura* – resulted in statistically higher CR and ERR than any of the other treatments. High ERRs are particular relevant for morbidity control.

Our results confirm previous findings reviewed by Olsen (2007), i.e., elevated efficacy against *T. trichiura* when albendazole was combined with ivermectin (Olsen, 2007), but contrast to findings from a Ugandan trial, where concurrent administration of ivermectin failed to enhance efficacy in curing *T. trichiura* infections in pregnant women (Ndyomugenyi et al., 2008). However, in the Uganda study, there were only 17 individuals infected with *T. trichiura*, hence results have to be interpreted with caution. Compared with other trials, we found considerably lower CRs and ERRs both with albendazole and mebendazole alone and an albendazole-ivermectin combination against *T. trichiura* (Olsen, 2007; Keiser and Utzinger, 2008; Ndyomugenyi et al., 2008). A plausible explanation is that we used a more rigorous diagnostic approach with 4 Kato-Katz thick smears examined before and after treatment. Indeed, CRs would have been considerably higher if only duplicate Kato-Katz thick smears were examined before and after treatment: 60% for mebendazole-ivermectin, 53% for, albendazole-ivermectin, 30% for mebendazole and 21% for albendazole. A potential onset of drug resistance should be kept in mind, not least because of high anthelmintic drug pressure in Zanzibar over the past 15 years (Albonico et al., 2003; Knopp et al., 2009; Stothard et al., 2009). The low efficacy of albendazole against *T. trichiura* in our study is worrying, also because a prior spot-check had revealed a low CR of 25% in preschool-aged children, using a less rigorous diagnostic approach (Stothard et al., 2009). While a trial conducted on the neighboring island Pemba in 1992/1993 had revealed similarly low CRs of albendazole and mebendazole against *T. trichiura* as found in our study, the ERR of albendazole against *T. trichiura* had been significantly higher (73%) (Albonico et al., 1994). An ERR <50% raises concern and should trigger more detailed investigations, e.g., *in vitro* tests for further clarification of whether resistance is emerging, as it has been previously suggested for schistosomiasis (WHO, 1999). With the exception of the egg hatch assay for

human hookworm (Albonico, 2003; Albonico et al., 2005), *in vitro* tests for the biological confirmation of anthelmintic resistance have yet to be developed for human nematode infections.

Compared with CRs of other treatment options against *T. trichiura*, e.g., combination therapies with pyrantel-oxantel (CR=32%) (Albonico et al., 2002) or mebendazole-levamisole (CR=23%), or treatment with levamisole (CR=10%) (Albonico et al., 2003) or tribendimidine alone (CR=0) (Steinmann et al., 2008), the CRs observed in our study for the two ivermectin combined therapies were considerably higher.

The small groups for assessment of treatment outcomes of the 4 treatment regimens against hookworm and *A. lumbricoides* resulting from low prevalences of co-infections are a limitation of our study. The highest decrease in both prevalence and infection intensity was achieved with albendazole regardless of whether it was combined with ivermectin, in line with previous findings (Marti et al., 1996; Keiser and Utzinger, 2008).

Regarding the helminth re-infection potential in Zanzibar, the follow-up period of 3-5 weeks post-treatment might be considered as another limitation of our study. Anthelmintic drug efficacy should be monitored 2-3 weeks post-treatment (Scherrer et al., 2009). However, an increase in time until the last follow-up stool sample after treatment was collected showed no association with *T. trichiura* infection in our study. Hence, potential re-infections should not have confounded our results.

The different treatments investigated here were safe, and AEs were transient and mostly mild. Nevertheless, compared to a previous review where only gastro-intestinal AEs occurred with a frequency >1% (Horton, 2000), we not only observed abdominal cramps and diarrhea, but most other solicited AEs showed frequencies above 1%. However, the frequency of AEs in our study might be overestimated, since we did not assess pre-treatment conditions, which might allow distinguishing between treatment-related and unrelated AEs. Importantly, the prevalence and intensity of soil-transmitted helminth infections in Zanzibar have significantly declined over the past 15 years (Knopp et al., 2009). Because there might be higher rates of AEs in populations with higher infection rates and no previous treatment history, similar studies are needed to ensure like for like comparisons.

Concluding, the addition of ivermectin to albendazole and mebendazole improves treatment outcomes against *T. trichiura* and will have an extra beneficial effect against an often co-endemic, but neglected soil-transmitted helminth, *Strongyloides stercoralis*, and against co-endemic ecto-parasites. These drug combinations might be considered in soil-transmitted helminth control programs and individual patient management.

16.6. Acknowledgments

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17. Discussion

This PhD thesis is incorporated in the nexus of the Swiss TPH built around the triangle of innovation, validation and application (Table 26). The thesis focuses on the epidemiology and control of soil-transmitted helminth infections on Unguja Island, which is the larger of the two major islands of Zanzibar, belonging to the United Republic of Tanzania. In the context of chemotherapy-based helminth control programmes implemented on Zanzibar since the mid-1990s, we were interested in the current prevalence and infection intensities of *A. lumbricoides*, hookworm, *T. trichiura* and *S. stercoralis*, and the underlying demographic, environmental, socio-economic and behavioural risk factors among school-aged children and entire populations both in rural and peri-urban settings. We also assessed the efficacy and safety of the drugs albendazole and mebendazole, which have been widely used in Zanzibar's helminth control programmes, and the effect of combination therapy with albendazole-ivermectin and mebendazole-ivermectin, placing particular emphasis on cure rates and egg reduction rates against *T. trichiura* infections. Noteworthy, to our knowledge, the combination of mebendazole plus ivermectin was investigated for the first time. Additionally, we compared different coprological methods and method combinations for the diagnosis of mainly low-intensity soil-transmitted helminth infections. For the first time, we used the recently developed FLOTAC technique for the diagnosis of *A. lumbricoides* and *T. trichiura* and were able to confirm its higher sensitivity when compared to the widely used Kato-Katz technique, as shown before for hookworm diagnosis. Finally, we transferred the FLOTAC method to the HCLU in Zanzibar and assessed its "field-application" and the potential for drug efficacy evaluation in comparison to the Kato-Katz method.

The findings of the cross-sectional parasitological and questionnaire surveys, and the randomized controlled trial carried out in the frame of this PhD in Unguja will be summarized and discussed in the following sections. Future research needs will be highlighted and practical implications to advance helminth control in Zanzibar will be outlined. The results of this thesis will be of relevance for soil-transmitted helminth control programmes in Zanzibar and might find application at a broader level elsewhere.

Table 26. Contribution of the different chapters of this PhD thesis to the nexus of the Swiss TPH built around the triangle of innovation, validation and application. STH: soil-transmitted helminth

Chapter	Title	Innovation	Validation	Application
9	Diagnosis of STH in the era of preventive chemotherapy: effect of multiple stool sampling and use of different diagnostic techniques			The effect of multiple stool sampling and combination of diagnostic methods on the sensitivity of the Kato-Katz, Baermann and Koga agar plate techniques was assessed in a low infection intensity area
10	A single FLOTAC is more sensitive than triplicate Kato-Katz for the diagnosis of low-intensity STH infections	The FLOTAC method was applied for the first time to diagnose <i>A. lumbricoides</i> and <i>T. trichiura</i> infections	The high sensitivity of FLOTAC for hookworm diagnosis was confirmed	
11	FLOTAC: a promising technique for detecting helminth eggs in human faeces		The sensitivity of FLOTAC for STH diagnosis in stool samples from Côte d'Ivoire and Zanzibar was reviewed and advantages and disadvantages of the method discussed	
12	Comparison of the Kato-Katz and FLOTAC techniques within an anthelmintic drug efficacy trial	Cure rates and egg reduction rates were determined for the first time with the FLOTAC method and compared with the Kato-Katz method in a drug efficacy study		The technicians of the HCLU were trained in the use of the FLOTAC method in Zanzibar
13	Spatial distribution of STH, including <i>S. stercoralis</i> , among children in Zanzibar			The epidemiology of STH with particular consideration of <i>S. stercoralis</i> was assessed in six districts on Unguja
14	Patterns and risk factors of helminth infections and anaemia in a rural and a peri-urban community in Zanzibar, in the context of helminth control programmes			Differences in the epidemiology and risk factors of STH infections were found when comparing a rural and a peri-urban community on Unguja
15	Changing patterns of STH in Zanzibar in the context of national helminth control programmes		Preventive chemotherapy significantly reduced the prevalence and intensity of STH infections. More sensitive tools or method combinations are necessary to document these changing patterns	
16	Albendazole and mebendazole administered alone or in combination with ivermectin against <i>T. trichiura</i> : a randomised controlled trial	The efficacy and safety of the combination of mebendazole plus ivermectin against <i>T. trichiura</i> and other STH was assessed for the first time	The high efficacy of the combination of albendazole plus ivermectin against <i>T. trichiura</i> was confirmed	The efficacy of single dose albendazole and mebendazole after a decade of large-scale drug administration in Zanzibar was assessed

17.1. The epidemiology of soil-transmitted helminth infections on Unguja

In the context of large-scale and sustained helminth control programmes, the epidemiology of the targeted helminth infections needs regular and careful reassessment. Our aim was to determine the current magnitude of soil-transmitted helminth infections on Unguja, where helminth control programmes emphasizing preventive chemotherapy primarily targeting school-aged children (against soil-transmitted helminthiasis and schistosomiasis) and whole communities (against lymphatic filariasis) are implemented since 1994 and 2001, respectively. We found that soil-transmitted helminth infections are still endemic on Unguja, but prevalence and infection intensities have declined. Our studies indicate that *T. trichiura* is currently the predominant species across the island, followed by hookworm, *A. lumbricoides*, and *S. stercoralis* (Knopp et al., 2008b; 2009a). The highest prevalence of soil-transmitted helminth infections is found in District North A (Knopp et al., 2008b; 2010b; Stothard et al., 2008). Concurrent infections with multiple soil-transmitted helminth species are still common, but infection intensities are generally low (Knopp et al., 2008b; 2009a; 2010b). Prevalence and infection intensities of *A. lumbricoides* and *T. trichiura* are highest in young children and decrease with increasing age (Knopp et al., 2010b). The pattern of hookworm and *S. stercoralis* infections is less clear cut (Knopp et al., 2010b). Besides demographic factors (i.e. age and sex), there are environmental factors such as underground and soil composition, vegetation and humidity, and behavioural factors such as eating raw vegetables or salad, not washing hands after defecation, or a recent travel history that were identified as risk factors for infection with certain soil-transmitted helminth species in Zanzibar (Knopp et al., 2008b; 2009a; 2010b; Stothard et al., 2008).

Our findings are based, in most instances, on a rigorous diagnostic approach, i.e. the examination of multiple stool samples per individual using a combination of different diagnostic techniques. All our data are derived from cross-sectional studies, including several hundred individuals from selected villages and peri-urban areas rather than from island-wide large-scale surveys. Hence, care is indicated when attempting to generalize observations made across Unguja.

In all our surveys we consistently found a high prevalence of *T. trichiura*, far higher than that of other soil-transmitted helminth species (Knopp et al., 2008b). What is the likely explanation of this observation? Data from the early/mid-1990s documented that all soil-transmitted helminths showed high prevalences and infection intensities (Marti et al., 1996; Montresor et al., 2001). Control programmes implemented over the past years were built

around preventive chemotherapy using either albendazole or mebendazole. Importantly both drugs are only moderately efficacious against *T. trichiura* whilst both drugs are highly efficacious against *A. lumbricoides* and albendazole is highly efficacious against hookworm (Keiser and Utzinger, 2008; Knopp et al., 2010a) (see chapter 17.2.2.).

It is conceivable that the high prevalence of all soil-transmitted helminth species revealed in the settings located in district North A is not only a result of individual risk factors, and favourable soil and vegetation conditions for egg and larvae development in this area (Knopp et al., 2008b), but might also mirror the generally poor hygienic situation in this district of Unguja (Figure 21). Almost half (46%) of the residents in district North A have no access to toilet facilities at all, the remaining part (50%) is mainly using traditional pit latrines, and only 3% have flush toilets or ventilated improved pit latrines (OCGS, 2007). Hence, environmental contamination with faeces containing soil-transmitted helminth eggs and larvae is expected to be high, abetting new infections as well as re-infections after successful treatment. The lower prevalence of soil-transmitted helminth infections observed in settings in the South and Central districts of Unguja, and particularly in the district Urban and the peri-urban district West, is possibly a result of both the better sanitary infrastructure in these areas, as well as more unfavourable environmental conditions for helminth ova and larvae development.

Despite the considerable number of people still infected with one or multiple species of soil-transmitted helminths on Unguja, the low infection intensities found in the surveyed settings are encouraging and imply that the morbidity caused by soil-transmitted helminth infections on Unguja is reduced in the face of preventive chemotherapy. Findings from the Philippines where concurrent low-intensity infections with multiple parasite species resulted in increased odds of having anaemia were luckily not confirmed in our study (Ezeamama et al., 2005).

The typical pattern of soil-transmitted helminth infections being most prevalent and intense in young children seems not to have changed on Unguja despite 15 years of helminth control activities emphasizing regular administration of anthelmintic drugs to at-risk groups such as school-aged children (Bundy et al., 1992; Bethony et al., 2006; Knopp et al., 2010b). Hence, preventive chemotherapy needs further consolidation and preschool children might be included in addition to the school-aged population. Since also personal behaviour plays an important role for soil-transmitted helminth infection (Raso et al., 2006; Stothard et al., 2009; Knopp et al., 2010b) there is additional need for improved health education at schools and in the communities, which finally needs to be consolidated by the construction of latrines and improved access to clean water. The impact of CLTS on soil-transmitted helminthiasis,

schistosomiasis and other neglected tropical diseases should be evaluated (Pattanayak et al., 2009; Utzinger et al., 2009).

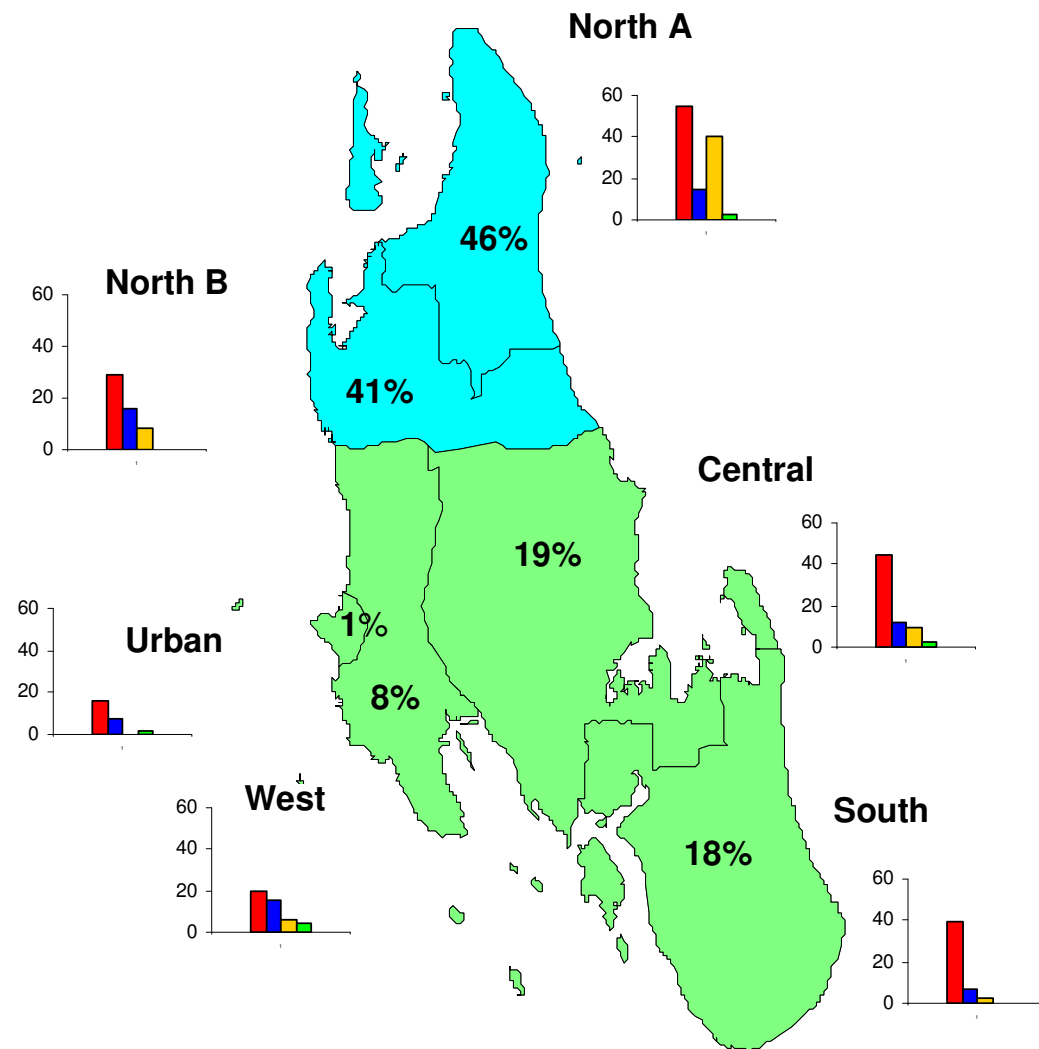


Figure 21. Percentage of the population of the 6 districts on Unguja with no toilet facilities and the prevalence of soil-transmitted helminth infections in one madrassa of district North A, B, Central, West, and South, and in 5 primary schools in the urban district (red bar: *T. trichiura*; blue bar: hookworm; orange bar: *A. lumbricoides*; green bar: *S. stercoralis*). Figure adapted from (OCGS, 2006)

17.2. Helminth control in Zanzibar

For a successful and sustainable reduction of infection intensities and prevalence, helminth control programmes need to be regularly revised and adapted to the current epidemiological situation of the targeted worms. This issue has been discussed in considerable detail for schistosomiasis control in China (Utzinger et al., 2005; Wang et al., 2008). We aimed to reveal the long-term success and shortcomings of helminth control programmes in Unguja placing emphasis on soil-transmitted helminthiasis, and use this opportunity to discuss key issues that will be important to render helminth control in Zanzibar more effective.

17.2.1. Success of soil-transmitted helminth control programmes and improved sanitation in Zanzibar

Studies implicit that the Zanzibar helminth control programmes, including *Piga vita kichocho* (primary emphasis on urinary schistosomiasis) as well as the GPELF (primary emphasis on lymphatic filariasis), have significantly reduced soil-transmitted helminth prevalence and infection intensities on Unguja (Mohammed et al., 2006; Knopp et al., 2009a; Stothard et al., 2009). The reduction of prevalence is most obvious for hookworm infections. While prevalences in district North A were reported to be 94% in two primary schools (Chaani and Kinyasini) surveyed in 1994 before the onset of the school-based helminth control programme in Unguja (Marti et al., 1996), and 78% in schoolchildren from 10 shehias surveyed in 1998 after 13 rounds of treatment (Montresor et al., 2001), the hookworm prevalence found in the studies of this PhD carried out between 2007 and 2009 ranged between 14% and 25% in three primary schools (Chaani, Kilombero and Kinyasini), all located in district North A (Knopp et al., 2009a; 2010a). Concurrently, a significant decline in the prevalence of *A. lumbricoides* infections was observed. School children surveyed in 1994 and 1998 had prevalences of 58% and 23%, respectively (Marti et al., 1996; Montresor et al., 2001), whereas in 2007, the prevalence of *A. lumbricoides* was 17% among children visiting the primary schools in Chaani and Kinyasini, and only 9% of the children from Kilombero and Kinyasini in 2009 (Knopp et al., 2009a; 2010a). Least obvious and hence worrying is the small decline in the prevalence of *T. trichiura*. In the 1990s, *T. trichiura* infected 91% and 73% of the surveyed schoolchildren, whereas we found prevalences that were still around 60% some 10-15 years later.

Not only the prevalence but most importantly the infection intensities of all major soil-transmitted helminth species have decreased significantly over the past decade. While in 1994

a third of the surveyed children had moderate or heavy infection intensities according to pre-set thresholds put forth by WHO (Table 1) (WHO, 2002a), only 10% of the schoolchildren carried moderate or heavy infections in 1998 (Marti et al., 1996; Montresor et al., 2001), and 95% of all infections detected in 2007-2009 were classified as light (Knopp et al., 2009a).

A strength of our own studies is that at least two stool samples per individual were examined with the Kato-Katz method. Only one stool sample per individual was investigated in the studies done by Marti and colleagues (1996) and Montresor and colleagues (2001). Multiple stool sampling is a simple means to enhance diagnostic sensitivity of direct techniques, and hence results in prevalence estimates that are closer to the 'true' values. Therefore, it is conceivable that the prevalence of soil-transmitted helminths derived in the 1990s was an underestimation of the 'true' situation, and that the decrease in prevalence was even steeper than reported here. Again, one has to consider that the results compared above were derived from a handful of settings and not from island-wide surveys. Nevertheless, they clearly indicate a trend of decreasing soil-transmitted helminthiases in Unguja, and confirm unpublished data from the MoHSW and a published report from the *Piga vita kichocho* programme (Mohammed et al., 2006; Stothard et al., 2009). Preventive chemotherapy, therefore, has been confirmed as a powerful tool to reduce prevalence and intensity of infection and hence morbidity (Savioli et al., 2002; 2009).

However, the success of reduced prevalence and intensities of soil-transmitted helminth infections on Unguja is probably only partially attributable to preventive chemotherapy. A reduction in poverty and an increase of households with toilet facilities has likely contributed to the observed decrease in soil-transmitted helminthiasis. For instance, the proportion of houses with toilet in Zanzibar increased from 49% in 1991 to 72% in 2004/05, and in rural areas from 31% to 59%, respectively (OCGS, 2006). In district North A, where the helminth data from above were derived, only 42% of the households had a toilet in 2002, whereas 54% of the households were using a toilet in 2004/05 (OCGS, 2007). It is therefore likely that the environmental contamination with faeces, and hence with soil-transmitted eggs or larvae decreased with increased toilet use over the past years, and in turn reduced the transmission of soil-transmitted helminths and the risk of new or repeated infections.

17.2.2. Drug efficacy and safety in Zanzibar and at broader levels

The application of anthelmintic drugs to high-risk groups will remain the cornerstone of helminth control in areas targeted for morbidity control and where limited human and

financial resources and poverty hinder the implementation of a sound hygienic infrastructure. The regular reassessment of the efficacy of drugs in long-term use and the development of new drugs or drug combinations is hence of prime importance to assure the progress of helminth control and to reduce the risk of resistance development (Albonico et al., 2004; Hotez et al., 2007b). Our aim was to assess the current efficacy of albendazole and mebendazole, two drugs that have been widely used in Zanzibar against soil-transmitted helminth infections. Additionally, in view of the unacceptable high prevalence of *T. trichiura* infections in Zanzibar after several years of large-scale drug administration, and triggered by recent reports and a meta-analysis documenting low efficacies of albendazole and mebendazole when used at standard single-dose regimens, but improved treatment outcomes of an albendazole plus ivermectin combination therapy (Olsen, 2007; Keiser and Utzinger, 2008), we were interested in investigating the cure rate and egg reduction rate not only of albendazole plus ivermectin but also of mebendazole plus ivermectin. To our knowledge, the combination of mebendazole plus ivermectin against soil-transmitted helminthiasis has not been investigated before.

We found that both albendazole and mebendazole either administered alone or in combination with ivermectin were still highly efficacious against *A. lumbricoides*, since egg reduction rates were above 99% (Knopp et al., 2010a). As revealed in earlier studies, albendazole was more efficacious than mebendazole against hookworm infections (Bennett and Guyatt, 2000; Flohr et al., 2007; Keiser and Utzinger, 2008), and the addition of ivermectin did not significantly improve the treatment outcomes. The cure rates and egg reduction rates of hookworm infections using albendazole (59% and 94%) or mebendazole (34% and 78%) are similar to results obtained in Pemba in 1992/93 (Albonico et al., 1994). Hence, we feel that there is no sign of resistance development with regard to *A. lumbricoides* and hookworm infections on Unguja. However, the cure rates of hookworm are unsatisfactory for both drugs if applied as single oral dose. A more sweeping approach to target hookworm infections could therefore be the application of albendazole over three consecutive days (Steinmann et al., 2011). The treatment outcomes of single-dose albendazole or mebendazole against *T. trichiura* infections were calamitous in our study (Knopp et al., 2010a). Especially the egg reduction rate of albendazole (40%) is worrying since, according to WHO, egg reduction rates below 50% should be considered as sign of resistance and need more detailed investigation (WHO, 1999). Ivermectin, however, added on the efficacy of both drugs, and the highest cure rate (55%) and egg reduction rate (67%) was achieved with the mebendazole plus ivermectin combination. Other treatment combinations, e.g. pyrantel in combination with

oxantel, had previously shown improved treatment outcomes against *T. trichiura* (cure rate: 38%; egg reduction rate: 87%) in comparison with single-dose drug administration, too (Albonico et al., 2002). However, also the efficacy of drug combinations against *T. trichiura* is rather unsatisfactory, and hence there is a need to develop new and more efficacious drugs against both hookworm and *T. trichiura*. A limitation of our study and of drug efficacy studies in general is that there is no evidence based and standardized protocols about when exactly after treatment the cure rate and egg reduction rate of the different helminth species should be assessed. Efficacy measured after 7 days might result in a different estimate of a treatment outcome than if measured after, say 21 days. Hence, studies in distinct settings endemic for soil-transmitted helminthiasis are needed to find out how many days or weeks after treatment egg shedding is at a minimal level. This information should be gathered through multi-centric trials and data analysed at a meta-level to put forward evidence-based recommendations on when exactly drug efficacy should be measured for specific soil-transmitted helminth species. A recent investigation focussing sequential helminth egg output after albendazole and praziquantel administration could serve as a template (Scherrer et al., 2009).

Similarly, the assessment of adverse events caused by anthelmintic drug application needs more standardization. Our study suggests that adverse events, despite being generally mild and transient, occur more frequently than reported in other studies (Horton et al., 2000; Knopp et al., 2010a). However, it is generally difficult to define in questionnaire interviews which complications are exclusively caused by treatment and which are ‘normal day’ interferences. It might hence be worth to develop standardized questionnaires applied a few days before and after treatment to distinguish between “general” and drug-related adverse events and to provide a more rigorous assessment of evidence. Noteworthy, Unguja is a setting where anthelmintic drugs have been administered for many years now and where infection intensities are low. It will be of prime importance to repeat a randomised controlled trial on the efficacy and safety of a mebendazole-ivermectin combination therapy in a setting with high infection intensities, particularly to assure that adverse events remain minor and transient in a “naïve” population hosting a high number of worms.

17.2.3. The need for advanced health education and improved environmental sanitation on Unguja and additional suggestions for a more effective soil-transmitted helminth control

Since infection intensities with soil-transmitted helminths on Unguja seem to have dropped to a low level, helminth control might now shift from morbidity control to prevalence and transmission control with the ultimate goal of elimination. To approach this goal, control measures consolidating regular anthelmintic treatment are needed. The WHO is emphasizing that anthelmintic treatment campaigns always need to be accomplished by efforts to improve information, education and communication (IEC), and water supply and sanitation (Montresor et al., 1998). While school-based and community-based treatment coverage rates in Zanzibar were high over the past decade (Mohammed et al., 2006; WHO, 2008) and a huge part of the population has access to improved water supplies (OCGS, 2006; Stothard et al., 2009), there seem to remain huge gaps in adequate health education, hygiene behaviour and appropriate sanitation. For long-term sustainability and further progress of helminth control in Zanzibar, these gaps need to be filled.

Hence, in addition to regular and area-wide preventive chemotherapy in schools and communities, the Zanzibar helminth control programme should implement a sound training schedule for locally involved health staff (e.g. health officers, nurses, and health education teachers) to guarantee a sound knowledge transfer to adults and children in endemic areas and to introduce a behavioural change in high-risk groups. It should also be part of the Zanzibar helminth programme to hold regular informative meetings with shehas and whole communities, particularly in the shehias with highest prevalences, to explain the transmission, burden and prevention of helminth infections. Appealing posters, leaflets, booklets and plays indicating risks for infection and appropriate behaviour should be created and widely disseminated. Famous people (e.g. the president or local rock stars) should promote appropriate hygienic behaviour in public speeches. Finally, the level of knowledge should be regularly tested by helminth control team members, e.g. on the days of treatment in schools by asking children about worm transmission cycles and preventive measures before deworming.

As soon as deprived communities are made aware of soil-transmitted helminthiasis and understand the importance of safe excreta disposal, their knowledge and motivation for behavioural change must be sustained by the provision of latrines and safe water (Albonico et al., 2006). The number of households using toilets has increased over the past years in Zanzibar (OCGS, 2007). However, yet only a tiny fraction of Zanzibar's rural dwellers has access to improved toilet facilities (OCGS, 2006). There is a general perception that the

construction of latrines is very expensive (Albonico et al., 2006). Hence, many communities or families cannot afford the construction of latrines or even the purchase of shoes, and hence it is poverty which hampers the effective control of soil-transmitted helminthiasis most. However, recent experiences with CLTS show that local communities can find their own way of implementing relatively inexpensive ways of sanitation. The impact of CLTS on the epidemiology of soil-transmitted helminth infections needs to be assessed.

The issue of poverty reduction goes beyond the action that can be taken by the Zanzibar helminth control programme but needs to be addressed by the Revolutionary Government of Zanzibar. A step towards poverty alleviation was made in 2002 by formulating the Zanzibar Poverty Reduction Plan (MFEA, 2002). Besides measures to reduce poverty by improving the economic, educational, transport, governmental and various other sectors, the Zanzibar Poverty Reduction Plan suggests the following actions to improve the water supply and sanitation system of Zanzibar: (i) to expand sanitation education through seminars, the mass media and drama, and political campaigns, (ii) to update the sanitation legislation, (iii) to review the options for water treatment and to propose those suitable for implementation, (iv) to construct treatment plants and to rehabilitate the existing drainage systems, and (v) to increase the number of households with latrines and septic tanks through introducing alternative building materials and methods, through establishing guidelines and rules for new houses, and through providing training on community construction and use of latrines and septic tanks. In context with the Development Vision 2020 for Zanzibar the following specific targets were set: (i) to provide 100% safe water in urban areas by 2020, and (ii) to provide 60% of rural households and 88% of urban households with toilet facilities by 2010. However, despite the encouraging goals set in the Zanzibar Poverty Reduction Plan, at the onset of the new millennium, the expansive implementation of the plan is challenged by insufficient funding, a lack of absorptive capacity (i.e. skilled people who are able and willing to do the work) and governance issues (ZPRP-PTF, 2002).

One additional suggestion of how to render helminth control in Zanzibar more effective is adopted from veterinary public health. In West Africa, egg excretion and transmission of a number of helminth species infecting cattle peak in the rainy season (Zinsstag et al., 1994). Hence, reducing the worm burden through anthelmintic treatment of cattle shortly before the rainy period starts is considered an effective measure to decrease helminth transmission and to increase health and therefore weight in cattle (Zinsstag et al., 1994; 2000). Soil moisture and atmospheric humidity are also known to influence the development and survival of human soil-transmitted helminth ova and larvae (Brooker et al., 2006). Higher humidity is associated

with faster development of eggs of *A. lumbricoides* and *T. trichiura*, and of larvae of hookworm (Spindler, 1929; Udonsi et al., 1980). Hence, it might be worth to find out about potential increased transmission patterns in Zanzibar and to plan the treatment of preschool and school-aged children or whole communities accordingly. If there are seasonal peaks of egg shedding, targeted treatment campaigns to cut them might be an effective way to advance helminth control in Zanzibar or elsewhere.

17.3. Soil-transmitted helminth diagnostics in the era of preventive chemotherapy

In Zanzibar the overall soil-transmitted helminth prevalence was still above 50% in many settings surveyed in the frame of this PhD. Hence, drug administration to at-risk groups, i.e. preschool children, school-aged children and women of child-bearing age, without prior diagnosis is still recommended, according to WHO guidelines (WHO, 2002a). However, the Zanzibar helminth control programme has successfully reduced soil-transmitted helminth prevalences and infection intensities and is crossing the line in the move from morbidity control to prevalence and transmission control (Mohammed et al., 2006; Knopp et al., 2009a; Stothard et al., 2009). Therefore, more targeted actions than regular drug administrations to at-risk groups without prior diagnosis will become an important issue in the agenda of Zanzibar's helminth control programme. Case management, i.e. the treatment of positive cases diagnosed by the health units, is recommended by WHO for communities with prevalences below 50% and low infection intensities (Montresor et al., 1998). To accurately interpret the current epidemiological situation in Zanzibar and to specifically plan future interventions, highly sensitive diagnostic tools are required. We aimed to assess the performance of multiple diagnostic tools in the era of preventive chemotherapy in Zanzibar. So far the Kato-Katz method (Katz et al., 1972) is routinely applied at HCLU for epidemiological assessments of soil-transmitted helminth infections and drug efficacy trials (WHO, 1996; Montresor et al., 1998). However, the sensitivity of copro-diagnostic methods such as the Kato-Katz method for the diagnosis of *A. lumbricoides*, *T. trichiura* and hookworm or the Koga agar plate (Koga et al., 1991) or Baermann method (García and Bruckner, 2001) for the detection of *S. stercoralis* is directly related to the number of helminth eggs or larvae excreted with the faeces (Hall, 1982; Pit et al., 1999; Knopp et al., 2008a). To reach an optimal sensitivity and to produce reliable results in settings with progressing helminth control marked by low infection intensities, these methods have to be prepared without compromise, and as ideal and close to recommendations as possible. The

sensitivity of the Kato-Katz method in our studies was increased, for example, when the slides were read for hookworm infections strictly within 20-30 min after preparation, but only after 3-5 hours for *A. lumbricoides* and *T. trichiura* eggs (Knopp et al., 2010c), as suggested by readily available guidelines offered by WHO (WHO, 1994), instead of reading the slides for all three major soil-transmitted helminth species at once 40-60 min after preparation (Knopp et al., 2008a), as often done because of time and cost saving reasons. The examination of multiple slides from the same stool sample and the collection of multiple consecutive stool samples per individual can additionally help to increase the sensitivity of a single method, and a combination of methods can further contribute to increase the overall negative predictive value, most likely by overcoming the effect of sporadic egg shedding and an uneven distribution of eggs in stool (Goodman et al., 2007; Knopp et al., 2008a; Steinmann et al., 2008). The collection of multiple stool samples per individual is, however, reducing the patient compliance and increasing the diagnosis-related costs (i.e. for transport and staff) (Knopp et al., 2010b). Hence, a highly sensitive diagnostic method is desired for application in large-scale epidemiological surveys of progressing helminth control programmes such as the one in Zanzibar. The target profile of such a diagnostic tool should, among other issues, be simple, fast and cheap, and able to detect multiple parasite species simultaneously at the same time. The newly developed FLOTAC technique (Cringoli et al., 2010) is currently discussed to meeting this goal. It has shown a higher sensitivity for the diagnosis of *A. lumbricoides*, hookworm and *T. trichiura* infections than multiple Kato-Katz thick smears in several studies (Uttinger et al., 2008; Glinz et al., 2009; Knopp et al., 2009b). The higher sensitivity of FLOTAC for the detection of hookworm was, however, not confirmed in our most recent investigations (Glinz et al., 2010; Knopp et al., 2010c). These discrepancies warrant further investigations and show that the FLOTAC method needs further validation in different epidemiological settings. Future investigations must reveal (i) what is the best preservation media and the appropriate length of storage for stool samples to be examined with the FLOTAC technique; (ii) which are the most appropriate and abundant flotation solutions for which parasite species; (iii) is there any flotation solution which is able to detect several helminth and intestinal protozoan species at the same time and with a high sensitivity; (iv) are the lower egg counts of the FLOTAC technique an underestimation or is the Kato-Katz method overestimating the true egg counts; (v) how much staff training is needed until the FLOTAC technique can produce reliable and comparable results if performed in multiple diagnostic centres; (vi) is the material needed for the FLOTAC technique available and affordable for helminth control programmes in resource-constraint countries; and (vii) at what

stage of helminth control becomes the FLOTAC technique a cost-effective diagnostic tool for large-scale programmes?

If, at one fine day in the near or far future, helminth control programmes in Zanzibar or elsewhere have reached the stage of transmission or even post-transmission control focussing elimination, there will be a need to adapt diagnostic methods beyond coprological egg detection. Tests suggested for this purpose include antibody detection via ELISA, and molecular marker identification tools such as the PCR (Bergquist et al., 2009). However, the ELISA tests used in the field studies of this PhD to diagnose *A. lumbricoides*, *S. stercoralis* and *S. haematobium* infections lacked sensitivity and particularly specificity (Knopp et al., 2010b). The reason was likely that antibody detection fails to distinguish between current and past infections, and because of a high cross-reactivity of antibodies developed against filarial or soil-transmitted helminth infections (Neppert, 1974; Bergquist et al., 2009). In settings with formerly high helminth prevalences, as found in Zanzibar, the ELISA technique might hence not meet the requirements for accurately detecting the last soil-transmitted helminthiasis cases. The PCR technique, in contrast, might become a reliable diagnostic tool to invent in, especially if stool instead of blood can be used for the detection of helminth DNA (Gasser et al., 2008; Verweij et al., 2007; 2009).

A reliable diagnosis of soil-transmitted helminth infections is also needed to accurately assess the efficacy of drugs regularly applied in helminth control programmes and hence for resistance monitoring. The study performed in the frame of this PhD comparing the drug efficacy determined with the Kato-Katz and FLOTAC method, revealed more conservative estimates of cure rates rates for all soil-transmitted helminths investigated after anthelmintic treatment when the FLOTAC technique was used (Knopp et al., 2010c). Despite the fact that FLOTAC is still under development for public health the results show that the current method of choice, namely the Kato-Katz method, overestimates the efficacy of drugs. This is an important finding, which needs additional investigation, since it implicates that the Kato-Katz method pretends cure at a level where it is not yet reached, which might lead to the termination of treatment before all worms have been killed, and is hence exacerbating resistance development.

17.4. Can lessons learned from Zanzibar be extrapolated to other helminth control programmes?

Nowadays the elimination for many tropical diseases is advocated. With regard to helminth infections it is the guinea worm whose eradication in the near future is most feasible (Lodge, 2010). Also lymphatic filariasis (Hooper et al., 2009), and onchocercosis (Thylefors and Alleman, 2006) are on the list to be eliminated as public health problems in the next 10 years (Gyapong et al., 2010). The elimination of schistosomiasis, however, will rather be a long-term process (King, 2009), with the exception of schistosomiasis japonicum in China (Wang et al., 2009a), and so will be the one of soil-transmitted helminthiasis.

Zanzibar, an island archipelago with clear boundaries, a strong public health system, and a long history of disease control programmes that sensitized the population for large-scale public health efforts (WHO, 2002b), could become the proof-of-concept if soil-transmitted helminth elimination is feasible at all in deprived settings. Parasitic disease control programmes in Zanzibar, including the one against malaria, were highly successful over the past years. Due to high treatment and insecticide treated bed net coverage rates, and additional other intervention measures, the number of reported malaria cases and deaths has decreased by more than 50% in Zanzibar over the past 10 years (WHO, 2009). Regular treatment of the whole eligible population over a period of five years has reduced the prevalence of microfilariae of lymphatic filariasis to zero at programme level (WHO, 2008), and also the prevalence of urinary schistosomiasis has dropped considerably over the past years due to intensive chemotherapy campaigns targeting school-aged children (Stothard et al., 2009). As discussed before, the control of soil-transmitted helminthiasis has now progressed from morbidity control to prevalence and transmission control and might, one day, reach the stage of elimination. It was outlined that the application of “preventive chemotherapy” alone will not meet this goal, since it might prevent morbidity but does not prevent the infection itself. The contamination of the environment by untreated “superspreaders” perpetuates the risk of infection also for treated individuals. Hence, to reduce transmission, to protect the uninfected and to finally eliminate soil-transmitted helminth infections, measures to improve health education and environmental sanitation have to be integrated in Zanzibar’s helminth control policy. Since these are more expensive than large-scale drug administrations, at least in the short term, funding and support by governmental and non-governmental organisations have to be secured. Noteworthy, improved sanitation would not only benefit soil-transmitted helminth control, but also the one of other water and sanitation related diseases, i.e. viral- or bacterial-caused diarrhoea, amoebiasis, schistosomiasis, and trachoma, and hence increased financial

expenses were highly profitable (Esrey et al., 1991). To render measures for the improvement of environmental sanitation effective, communities have to be integrated in decision making and planning and must understand the underlying importance of hygiene for health (Aagaard-Hansen et al., 2009; Smits, 2009; Wang et al., 2009b). It is in this context that CLTS should be tested and its impact on soil-transmitted helminthiasis and other neglected diseases evaluated. Finally, continued epidemiological surveillance using sensitive diagnostic tools adapted to the current stage of control will be necessary to reliably assess the current infection levels (Bergquist et al., 2009).

In view of the ongoing upscale of helminth control programmes the world over (Hotez et al., 2007a), the question arises whether lessons learnt from the microcosm of Zanzibar can be readily transferred to other soil-transmitted helminth programmes on the macrocosm mainland Africa or other continents. Similar to Zanzibar, soil-transmitted helminth control programmes in China, Korea, Lao People's Democratic Republic, Turkey and Uganda using regular large-scale anthelmintic treatment in line with health education and improved sanitation were able to reduce soil-transmitted helminth infection intensities, and hence mitigated the burden of morbidity (Hong et al., 2006; Ulukanligil, 2006; Kabatereine et al., 2007; Phommasack et al., 2008; Wang et al., 2009b; Zheng et al., 2009). However, large-scale treatment campaigns as well as access to safe water and sanitation become difficult in remote areas, and in regions where there is a high level of nomadism, civil unrest, political instability or natural disasters. The success of preventive chemotherapy programmes can be hampered by the unreasonableness of people to take drugs against a disease they do not see or feel, or by the fear of drug-related adverse events (Parker et al., 2008). The success of latrine construction and use, and of the establishment of sewage canals can be hindered by the tradition of night-soil use as fertilizer or by the use of wastewater for crops in some countries (Ulukanligil et al., 2001; Yajima et al., 2009). This problem can be mitigated by training people in special composting methods to kill soil-transmitted helminth eggs and other infectious agents in faeces before using it as fertilizer, by the alternative provision of cheap chemical fertilizers for agriculture, and by closed sewage canalisation (IWMI & SANDEC, 2002; Gallizzi, 2003; Hong et al., 2006; Wang et al., 2009b).

To say the least, soil-transmitted helminth control in whatever setting can only be successful and sustainable if (i) health education is provided to endemic communities to enhance their knowledge on diseases transmission and prevention, and hence to provoke improved personal and domestic hygiene, including toilet use, and to generate the acceptance of anthelmintic drugs resulting in a higher treatment coverage; (ii) the epidemiological

situation is accurately assessed using sensitive diagnostic tools, and if anthelmintic treatment is provided to endemic communities to mitigate infection associated morbidity and to reduce the egg shedding by infected individuals; and (iii) environmental sanitation, including convenient latrine construction, access to safe water and sewage systems, is provided at a high coverage rate and can be sustained due to affordability, availability at the local market and compatibility with local technologies (Figure 22) (Esrey et al., 1991; Asaolu and Ofoezie, 2003).

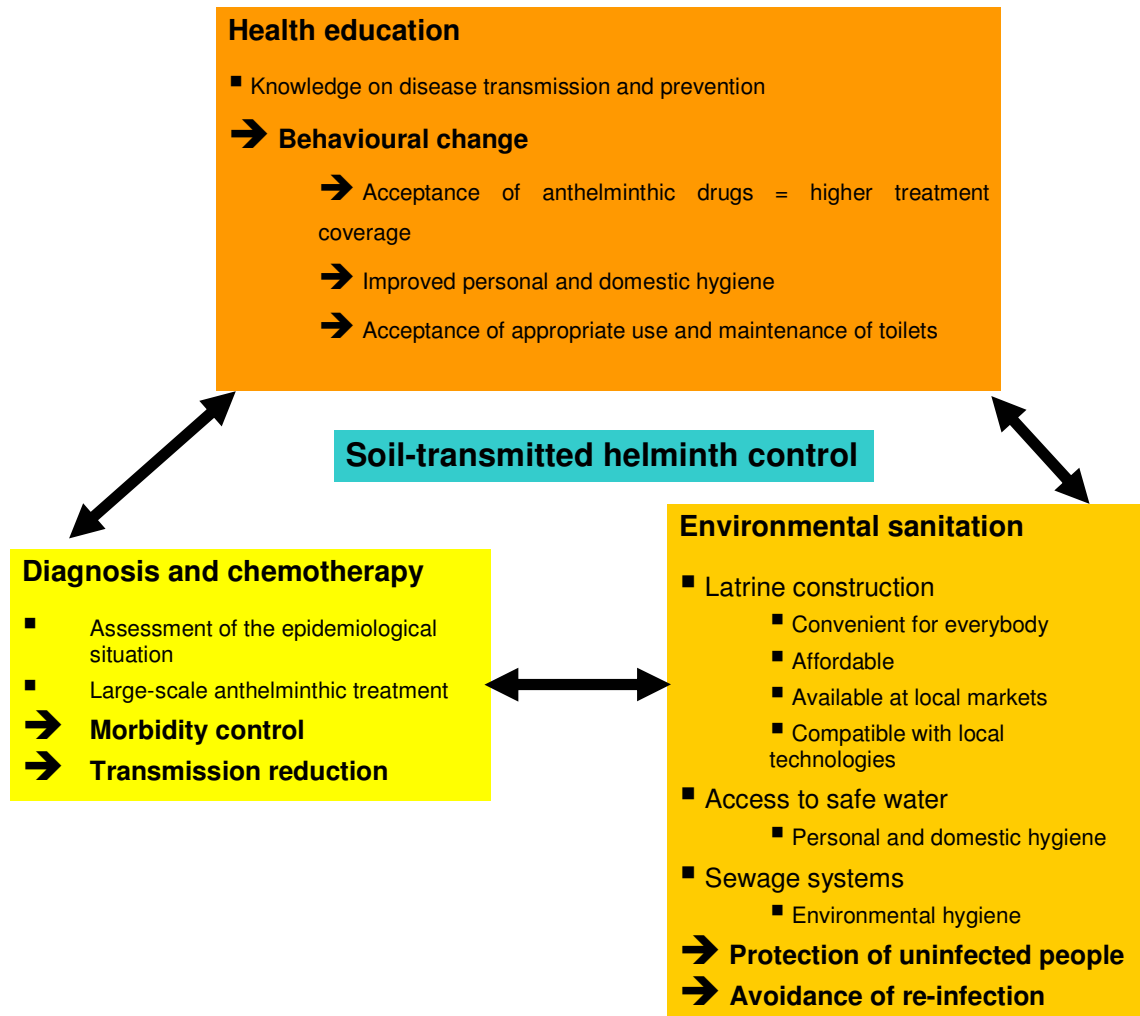


Figure 22. Nexus of soil-transmitted helminth control

17.5. Conclusion

The following set of conclusions is offered for consideration:

- Soil-transmitted helminthiases are still endemic on Unguja despite the implementation of large-scale helminth control programmes built around preventive chemotherapy. We found local idiosyncrasies, explained by demographic, socio-economic and environmental risk factors.
- *T. trichiura* is the predominant soil-transmitted helminth species, followed by hookworm, *A. lumbricoides* and *S. stercoralis*. Drugs with a high efficacy against *T. trichiura* and hookworm are yet to be developed, and hence altered application of albendazole and mebendazole should be considered to enhance treatment outcomes against either species and to lower the risk of resistance development.
- Albendazole or mebendazole combined with ivermectin enhances treatment outcomes against *T. trichiura*. Such combined therapies with ivermectin would have a fringe benefit in targeting *S. stercoralis* and ecto-parasite infections and possibly avert or delay the onset of a benzimidazole resistance.
- Consolidation and further advances in helminth control in Zanzibar will require thinking and acting beyond preventive chemotherapy. Health education measures, complemented with rigorous improvements in the sanitary infrastructure on the island are necessary. Improved sanitation will not only contain soil-transmitted helminth infections but additionally decrease intestinal protozoa, bacterial and viral infections causing diarrhoea and other symptoms associated with high morbidity and mortality.
- Sensitive diagnostic tools will become more and more important as helminth control moves ahead towards transmission control and elimination. As long as there are no field-applicable alternatives to the Kato-Katz method, it needs to be performed as close to WHO recommendations as possible. Multiple stool sampling and the combination of different methods can further increase diagnostic sensitivity.
- The FLOTAC technique developed by veterinary parasitologists and here extended to human public health has a higher sensitivity for the diagnosis of low-intensity soil-transmitted helminth infections than the Kato-Katz method, but some conflicting results were reported. There is a need for further evaluation and standardization of this technique before it might be recommended as a tool for monitoring helminth control programmes.

We are convinced that concerted efforts of (i) accurate epidemiological assessment using sensitive diagnostic tools, (ii) the choice of appropriate drugs or drug combinations according to the local parasite spectrum, and (iii) the integration of perspicuous health education and CLTS will contribute significantly to the progress and success of soil-transmitted helminth control in Zanzibar and elsewhere, and hence to the accomplishment of the millennium development goals.

17.6. Research needs and recommendations

17.6.1. Identified research needs

17.6.1.1 Soil-transmitted helminth control

- Developing new, efficacious, and safe drugs against *T. trichiura* and hookworm.
- Conducting a randomized controlled trial on the efficacy and safety of a mebendazole-ivermectin combination against *T. trichiura* (and other soil-transmitted helminths) in an area with moderate to high infection intensities to verify the results from Unguja.
- Investigating whether the *T. trichiura* population in Zanzibar is carrying molecular markers that point toward an emergence of benzimidazole resistance.
- Determinating of how many days after anthelmintic drug application the egg shedding of different soil-transmitted helminth species has reached a minimum in distinct settings; and developing evidence-based guidelines about when to assess drug efficacy.
- Conducting studies on adverse events using standardized questionnaires applied before and after treatment to distinguish treatment-related adverse events from normal day interferences.
- Assessing the impact of school-based health education on the prevalence of soil-transmitted helminth infections in schoolchildren.
- Assessing the impact of CLTS on the prevalence of soil-transmitted helminth infections and other neglected tropical diseases in communities, including costs and cost-effectiveness comparisons with preventive chemotherapy.
- Investigating over several years if there are seasonal peaks in egg shedding of soil-transmitted helminth species.
- Investigating whether deworming just before the season of increased egg shedding is reducing soil-transmitted helminth transmission within several years.

17.6.1.2 Diagnosis of soil-transmitted helminth infections

- Development of a cheap, simple and robust diagnostic tool that detects low-intensity soil-transmitted helminth infections with a high sensitivity.
- Further evaluation of the FLOTAC method, including:
 - most suitable preservation media and appropriate length of storage for stool samples;
 - most appropriate and abundant flotation solutions for single parasite species;
 - flotation solution, which is able to detect several helminth and intestinal protozoan species at the same time and with a high sensitivity;
 - in-depth investigation whether egg counts of the FLOTAC technique are an underestimation or if the Kato-Katz method is overestimating the ‘true’ egg counts;
 - how much staff training is needed until the FLOTAC technique can produce reliable and comparable results if performed in multiple diagnostic centres;
 - appraisal of material needed for the FLOTAC technique according to local availability and affordability for helminth control programmes in resource-constraint countries; and
 - determination at what stage of helminth control the FLOTAC technique becomes a cost-effective diagnostic tool.

17.6.2. Recommendations for helminth control in Zanzibar

- Continue regular treatment of preschool and school-aged children.
- Consider combination therapy with ivermectin.
 - to enhance treatment efficacy against *T. trichiura*;
 - to simultaneously target *S. stercoralis* and ecto-parasites; and
 - to delay the risk of development of benzimidazole resistance.
- Alternate the use of albendazole and mebendazole.
 - to target both hookworm and *T. trichiura*.
- Complement preventive chemotherapy with locally adapted health education and improvements in environmental sanitation.
- Implement regular training on the topic of helminth control for locally-involved health staff (e.g. health officers, nurses, and health education teachers).
- Hold regular informative meetings with community leaders and whole communities and explain the transmission, burden and prevention of helminth infections.
- Create and disseminate appealing posters, leaflets, booklets and plays indicating risks for infection and appropriate behaviour.
- Famous people (e.g. the president or local rock stars) should promote appropriate hygienic behaviour in public speeches.
- Test the degree of successful knowledge transfer regularly on the days of treatment in schools by asking children about worm transmission cycles and preventive measures before deworming campaigns.
- Ensure the full implementation of the Zanzibar Poverty Reduction Plan (ZPRP-PTF, 2002) including
 - expansion of sanitation education through seminars, the mass media and drama, and political campaigns;
 - update of the sanitation legislation;
 - review of options for water treatment and the proposal of those suitable for implementation;
 - construction of treatment plants and the rehabilitation of the existing drainage systems;
 - increase of the number of households with latrines and septic tanks through
 - introducing alternative building materials and methods,
 - establishing guidelines and rules for new houses, and

- providing training on community construction and use of latrines and septic tanks.
- Assess the effect of CLTS on the epidemiology of soil-transmitted helminthiasis and other diseases in Zanzibar.
- Ensure a sensitive diagnosis of soil-transmitted helminth infections by
 - a rigorous performance of the Kato-Katz method according to WHO bench aids (WHO, 1994);
 - multiple stool sampling; and
 - combination of diagnostic methods.

17.7. References

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18. Curriculum vitae**Personal information**

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Address	Mühlenrain 5 79576 Weil am Rhein, Germany
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Nationality	German
Date and place of birth	17/04/1979, Heidelberg, Germany
Languages	German (Mother tongue), English (fluent); French (good)

Education and work experience

04/2007 – 03/2010	PhD in Epidemiology, Swiss Tropical and Public Health Institute (Swiss TPH), University of Basel, Basel, Switzerland
PhD thesis	Diagnosis, epidemiology and control of soil-transmitted helminth infections in Zanzibar, Tanzania Supervision: Prof. Dr. Jürg Utzinger (Swiss TPH), Dr. Hanspeter Marti (Swiss TPH), Dr. David Rollinson (Natural History Museum London) Field stay: 7 months Zanzibar, Tanzania
11/2009	Supervisor of laboratory work in Tajikistan, for the UNICEF nutritional survey Tajikistan 2009 Fieldstay: 1 month Dushanbe, Tajikistan
10/2001 - 11/2005	MSc (Diploma) in Biology, Institute for Tropical Medicine and Hygiene, University of Tübingen, Tübingen, Germany
MSc Thesis	Antibody and cytokine responses in <i>Dracunculus medinensis</i> patients at distinct states of infection Supervision: Prof. Dr. Peter Soboslay, Prof. Dr. Hartwig Schulz-Key Fieldstay: 3 months Sokode, Togo
05/2003 - 08/2003	Summer Student at the Institute of Immunology and Infection Research, The University of Edinburgh, Edinburgh, UK Supervision: Prof. Dr. Rick Maizels
05/2002 - 08/2002	Research Assistant at the Institute for Tropical Medicine and Hygiene, Tübingen, Germany
10/1999 - 09/2001	BSc (Intermediate Diploma) in Biology, University of Göttingen, Göttingen, Germany
09/1998 - 03/1999	Laboratory Internship at the Comboni-Clinic, Sogakofe, Ghana
09/1989 - 06/1998	High School Bunsen Gymnasium, Heidelberg, Germany
Emphasis	Latin
09/1986 – 08/1989	Primary School Neuberg Grundschule, Dossenheim, Germany

Oral presentations at scientific meetings

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| 08/08/2009 | Talk at the European Congress of the Tropical Medicine and International Health (2009): "Efficacy of albendazole and mebendazole alone or in combination with ivermectin against <i>Trichuris trichiura</i> and other soil-transmitted helminths" (Verona, Italy). |
| 18/12/2008 | Talk at the research in progress meeting (2008) of the RSTMH (Royal Society of Tropical Medicine and Hygiene): "A single FLOTAC is more sensitive than multiple Kato-Katz thick smears for diagnosing low-intensity soil-transmitted helminth infections" (London, UK). |
| 23/10/2008 | Talk at the annual PhD-student meeting (2008) of the SSTMP (Swiss Society for Tropical Medicine and Parasitology): "Diagnosis of soil-transmitted helminthiasis among schoolchildren in Zanzibar in the context of the national helminth control programme" (Vevey, Switzerland). |
| 25/04/2008 | Talk at a meeting about diagnostic methods for soil-transmitted helminth infections (2008) at the WHO (World Health Organization): "Soil-transmitted helminthiasis with particular consideration to strongyloidiasis, among schoolchildren in Zanzibar in the context of the national helminth control programme" (Geneva, Switzerland). |
| 01/04/2008 | Talk at the Spring meeting (2008) of the BSP (British Society of Parasitology): "Effects of multiple stool sampling and different diagnostic techniques for the detection of soil-transmitted helminths" (Newcastle, UK). |
| 05/12/2007 | Talk at the annual PhD-student meeting (2007) of the SSTMP (Swiss Society for Tropical Medicine and Parasitology): "Soil-transmitted helminthiasis with particular consideration to strongyloidiasis, among schoolchildren in Zanzibar" (Münchenwiler, Switzerland). |
| 08/03/2006 | Talk at the annual meeting (2006) of the DTG (German Association of Tropical Medicine and International Health): "The cellular immune response and antibody reactivity of people infected with <i>Dracunculus medinensis</i> " (Tübingen, Germany). |

Teaching experience

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| 29/01/2010 | " <i>Dracunculus medinensis</i> : the eradication initiative" at the Short Course 'Disease Control' of the Master Course Programme 2010 of the Tropical Institute Heidelberg, Germany. |
| 11/12/2009 | "Water and Excreta related diseases" at the CINFO Course 2009 of the Swiss TPH, Basel, Switzerland. |
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2009/2010	Burckhardt Stiftung Basel – Personal stipend for the 3 rd year of the PhD (CHF 12,000).
2009	University of Basel – Travel Award for the European Congress of Tropical Medicine and International Health (CHF 448).
2009	Commission for Research Partnerships with Developing Countries (KFPE) through the SDC sponsored Jeunes Chercheurs Programme – Project Grant for Zanzibar 2009 (CHF 34,000).
2008	University of Basel – Travel Award for the RSTMH Research in Progress meeting (CHF 250)
2008	Swiss Academy of Sciences (Kommission für Reisestipendien der Akademie für Naturwissenschaften Schweiz SCNAT+) – Project Grant for Zanzibar 2008 (CHF 4,020)
2008	BSP – Travel Award for the BSP Spring meeting (£ 200)
2007	Technical Services Agreement from the WHO for fieldwork in Zanzibar 2007 (US\$ 7,300)
Prices	
2009	Price for the second best presentation at the European Congress of Tropical Medicine and International Health, Verona, Italy (€ 1,000).
2008	Price for the second best presentation at the RSTMH Research in Progress meeting, London, UK (£ 150).
Membership	
2008 - present	Member of the British Society for Parasitology (BSP)

19. Publications

Knopp, S., Amegbo, I.K., Hamm, D.M., Schulz-Key, H., Banla, M., Soboslay, P.T., (2008). Antibody and cytokine responses in *Dracunculus medinensis* patients at distinct states of infection. *Trans R Soc Trop Med Hyg* 102, 277-283.

Knopp, S., Mgeni, A.F., Khamis, I.S., Steinmann, P., Stothard, J.R., Rollinson, D., Marti, H., Utzinger, J., (2008). Diagnosis of soil-transmitted helminths in the era of preventive chemotherapy: effect of multiple stool sampling and use of different diagnostic techniques. *PLoS Negl Trop Dis* 2, e331.

Knopp, S., Mohammed, K.A., Khamis, I.S., Mgeni, A.F., Stothard, J.R., Rollinson, D., Marti, H., Utzinger, J., (2008). Spatial distribution of soil-transmitted helminths, including *Strongyloides stercoralis*, among children in Zanzibar. *Geospat. Health* 3, 47-56.

Knopp, S., Glinz, D., Rinaldi, L., Mohammed, K.A., N'Goran, E.K., Stothard, J.R., Marti, H., Cringoli, G., Rollinson, D., Utzinger, J., (2009). FLOTAC: a promising technique for detecting helminth eggs in human faeces. *Trans R Soc Trop Med Hyg* 103, 1190-1194.

Knopp, S., Mohammed, K.A., Rollinson, D., Stothard, J.R., Khamis, I.S., Utzinger, J., Marti, H., (2009). Changing patterns of soil-transmitted helminth infections in Zanzibar in the context of national control programs. *Am J Trop Med Hyg* 81, 1071-1078.

Knopp, S., Rinaldi, L., Khamis, I.S., Stothard, J.R., Rollinson, D., Maurelli, M.P., Steinmann, P., Marti, H., Cringoli, G., Utzinger, J., (2009). A single FLOTAC is more sensitive than triplicate Kato-Katz for the diagnosis of low-intensity soil-transmitted helminth infections. *Trans R Soc Trop Med Hyg* 103, 347-354.

Stothard, J.R., Sousa-Figueiredo, J.C., Standley, C., Van Dam, G.J., **Knopp, S.**, Utzinger, J., Ameri, H., Khamis, A.N., Khamis, I.S., Deelder, A.M., Mohammed, K.A., Rollinson, D., (2009). An evaluation of urine-CCA strip test and fingerprick blood SEA-ELISA for detection of urinary schistosomiasis in schoolchildren in Zanzibar. *Acta Trop* 111, 64-70.

Knopp, S., Mohammed, K.A., Stothard, J.R., Khamis, I.S., Rollinson, D., Marti, H., Utzinger, J., 2010. Patterns and risk factors of helminthiasis and anemia in a rural and a peri-urban community in Zanzibar, in the context of helminth control programs. *PLoS Negl Trop Dis* 4, e681.

Knopp, S., Mohammed, K.A., Speich, B., Hattendorf, J., Khamis, I.S., Khamis, A.N., Stothard, J.R., Rollinson, D., Marti, H., Utzinger, J., 2010. Albendazole and mebendazole administered alone or in combination with ivermectin against *Trichuris trichiura*: a randomized controlled trial. *Clin Infect Dis* 51, 1420-1428.

Glinz, D., Silué, K.D., **Knopp, S.**, Lohourignon, K.L., Yao, P.K., Steinmann, P., Rinaldi, L., Cringoli, G., N'Goran, E.K., Utzinger, J., 2010. Comparing diagnostic accuracy of Kato-Katz, Koga agar plate, ether-concentration, and FLOTAC for *Schistosoma mansoni* and soil-transmitted helminths *PLoS Negl Trop Dis* 4, e754.

Knopp, S., Speich, B., Hattendorf, J., Rinaldi, L., Mohammed, K.A., Khamis, I.S., Mohammed, A.S., Albonico, M., Rollinson, D., Marti, H., Utzinger, J., 2011. Diagnostic accuracy of Kato-Katz and FLOTAC for assessing anthelmintic drug efficacy. *PLoS Negl Trop Dis* 5, e1036.